Shri Mohan Jain
P. M. Priyadarshan
EDITORS

Breeding Plantation Tree Crops

Temperate Species

Springer

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S. Mohan Jain · P.M. Priyadarshan
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Editors
S. Mohan Jain
Helsinki University
Helsinki, Finland
jain@helsinki.fi

P.M. Priyadarshan
Rubber Research Institute of India
Regional Station
Agartala, India
rriipriya@rediffmail.com

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Preface

Tree species are indispensably supportive to human life. Due to their long life cycle and environmental sensitivity, breeding trees to suit day-to-day human needs is a formidable challenge. Whether they are edible as apple, cocoa, mango, citrus, litchi, pear, dates, and coconut or industrially essential as rubber or beverages like coffee and tea, improving yield under optimal, suboptimal, and marginal areas calls for a unified effort from scientists around the world. While the uniqueness of coconut as '*kalpavriksha*' (Sanskrit-meaning tree of life) makes its presence in every continent from Far East to South America, tree crops like cocoa, oil palm, rubber, apple, peach, grapes, and walnut prove their environmental sensitivity toward tropical, subtropical, and temperate climates. Desert climate is quintessential for date palm. Thus, from soft drinks to breweries to beverages to oil to tyres, the value addition offers a spectrum of products to human kind, enriched with nutritional, environmental, financial, social, and trade-related attributes.

Taxonomically, tree crops never confine to few families, but spread over a cross-section of genera, an attribute so unique that contributes immensely to biodiversity even while cultivated on commercial scale. Many of these species encourage other flora to nurture in their vicinity, thus ensuring their integrity toward preserving biodiversity. While wheat, rice, maize, barley, soybean, cassava, and banana make up the major food staples, many fruit-yielding tree species contribute toward nutritional enrichment in human life. The edible part of these species is the source of several nutrients that make additives for the daily human diet, for example, vitamins, sugars, aromas, and flavor compounds, and raw material for food-processing industries. Tree crops face an array of agronomic and horticultural problems in terms of propagation, yield, appearance, quality, diseases and pest control, abiotic stresses, and poor shelf life.

Shrinkage of cultivable land and growing demand has enforced these crops to be grown under marginal conditions that call for concerted efforts from breeders to improve these crops substantially. Concerted efforts have not been incurred to bring out the compilation of research done on tree crops grown under both traditional and nontraditional environments. Even if available, they

are scattered and also lack comprehensive treatment and wholesomeness. The task of improving yield in tree crops is foremost in the acumen of global agricultural research, and the advancements made in this arena are immense both at conventional and molecular means. This two-volume series on *Breeding Plantation Tree Crops* dealing both tropical and temperate species separately is a sincere effort toward compiling the research available worldwide and bring them to the reference of scientists, researchers, teachers, students, policy makers, and even planters. It is worthwhile to note that in the forthcoming years, tree crops are to be given much importance on par with annual crops due to carbon trading and nutritional upgradation of the daily diet.

Since tropical species are more diverse, the first volume on tropical species contains 16 chapters on fruits and nuts (banana, mango, guava, papaya, grape, date palm, litchi, avocado, and cashew), oil crops (coconut, oil palm, and olive), industrial crops (rubber), and beverages (coffee, tea, and cocoa). The second volume contains temperate fruit species including apple, apricot, almond, citrus, pear, plum, raspberry, and walnut.

The chapters were authored by crop-specific experts worldwide. We appreciate the untiring efforts rendered by the authors in ensuring the inclusion of latest advancements and their cooperation in revising their manuscripts timely. A few reviewers spared their precious time in improving the quality manuscripts. We are immensely thankful to them for their valuable help. Finally, we thank Springer for bringing out this series to the readers.

Helsinki, Finland — S. Mohan Jain
Agartala, India — P.M. Priyadarshan

Contents

Contributors

Manfred Fischer
BAZ Quedlinburg, Institut für Obstzüchtung Dresden-Pillnitz; IPK Gatersleben, Genbank Obst Dresden-Pillnitz (former affiliation), Söbrigener, Str. 15, D - 01326 Dresden, Germany, manfred.fischer@sz-online.de

Fred G. Gmitter
Institute of Food and Agricultural Sciences, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred FL 33850, USA, fgg@crec.ifas.ufl.edu

Thomas M. Gradziel
Department of Plant Sciences, University of California, Davis, California, tmgradziel@ucdavis.edu

Julie Graham
Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

Hannél Ham
ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

Walter Hartmann
Institute of Special Crop Cultivation and Crop Physiology, University of Hohenheim, Emil-Wolff-Straße 25, 70599 Stuttgart, Germany, walthart@uni-hohenheim.de

Nikki Jennings
Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

Chuck Leslie
Department of Plant Sciences, Mail stop 2, One Shields Ave., University of California, Davis, CA 95616, USA

Gale Mcgranahan
Department of Plant Sciences, Mail stop 2, One Shields Ave., University of California, Davis, CA 95616, USA, ghmcgranahan@ucdavis.edu

Michael Neumüller
Technical University of Munich, Center of Life and Food Sciences Weihenstephan, Unit of Fruit Science, Alte Akademie 16, 85354 Freising, neumueller@obstzentrum.de

S. Pereira-Lorenzo
Escola Politécnica Superior, Departamento Producción Vegetal, Universidad de Santiago de Compostela, Campus de Lugo, 27002, santiago.pereira.lorenzo@usc.es

A.M. Ramos-Cabrer
Escola Politécnica Superior, Departamento Producción Vegetal, Universidad de Santiago de Compostela, Campus de Lugo, 27002

Madhugiri Nageswara Rao
Institute of Food and Agricultural Sciences, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred FL 33850, USA

Jaya R. Soneji
Institute of Food and Agricultural Sciences, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred FL 33850, USA

Almond (*Prunus dulcis*) Breeding

Thomas M. Gradziel

1 Introduction

An adaptation to harsh climates combined with an ability to develop a deep and extensive root system has allowed cultivated and wild almond to exploit a wide variety of ecological niches in its ancestral range in central Asia extending from the Takla Makan desert in western China to the Mediterranean (Kester et al. 1991; Ladizinsky 1999). Almond is also well adapted to mild winter and dry, hot summer conditions due to its low chilling requirement for early bloom, rapid early shoot growth, and high tolerance to summer heat and drought. It is the earliest temperate tree crop to bloom, which limits production to areas relatively free from spring frosts. Because almond is self-sterile, it requires cross-pollination that further acts to promote genetic variability and, therefore, adaptability to new environments.

Commercial production is often limited by the need for cross-pollination in orchard systems, particularly in areas where spring storms can reduce both flowering duration and activity of required insect pollinators. A high susceptibility to fungal and bacterial diseases of the blossoms, leaves, branches, and fruits also reduces production in areas with rain and/or high humidity during the growing season (Kumar and Uppal 1990; Ogawa and English 1991). Similarly, excessive moisture in the root zone can result in tree losses due to root rots or asphyxia.

1.1 Origin and History

Early researchers proposed that cultivated almond resulted from selection from within a species listed originally as *Amygdalus communis* L. (syn. *Prunus communis* Archang.) based on studies of two natural populations originally

T.M. Gradziel
Department of Plant Sciences, University of California, Davis, California, USA
e-mail: tmgradziel@ucdavis.edu

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identified as *A. communis* and containing large numbers of sweet seeded individuals rather than the bitter kernels typically found in the wild (Watkins 1979). One population is located in the Kobet Dag mountain range in central Asia between present-day Iran and Turkmenistan, and the second population occurs on the lower slopes of the Tian Shan mountains between Kyrgyzstan and western China. The natural range of *A. communis* was proposed to have extended across Iran, the Transcaucausus, and eastern Turkey, and into present-day Syria, and thus overlapped with known sites of early almond cultivation (Denisov 1988; Kester et al. 1991). According to this view, the distinction between cultivated and wild forms gradually disappeared with direct and indirect human selection. However, because the purportedly natural sweet-kernelled populations closely resemble the phenotypic range of present-day cultivated almonds, it has recently been suggested that the Kobet Dagh and Tian Shan populations are, in fact, more recent remnants or escapes from later domesticated or semidomesticated orchards (Ladizinsky 1999). The emerging consensus is that cultivated almond represents a generalized, fungible kernel phenotype, possibly derived from *P. fenzliana*, but with contributions through natural interspecific cross-hybridizations with a range of related species occurring naturally within this range (Fig. 1), including *P. bucharica, P. kuramica,* and *P. triloba* (Godini 2000; Grasselly and Crossa Raynaud 1980; Kester et al. 1991; Socias i Company 2002).

A subsequent and widespread dispersal of 'cultivated' almonds occurred in three stages: Asiatic, Mediterranean, and Californian. The Asiatic stage included the initial domestication and the subsequent spread throughout central and southwestern Asia often along major prehistoric trade routes. The range centers on present-day Iran extending east to western China, northwest India, northern Pakistan, northwest through Turkey, and southwest into the uplands and deserts of central Israel and Syria. Almonds are reported in Hebrew literature as early as 2000 BCE. Their culture continues to the present time within the Asiatic region, where in many areas, almonds are grown under dryland, subsistence agricultural practices similar to those used thousands years ago.

In the Mediterranean stage, almonds appear to have been brought into Greece prior to 300 BCE, eventually being introduced to all compatible areas of the Mediterranean. Initial introductions may have come from the early ocean trading Phoenicians and Greeks during establishment of colonies in Sicily and other Mediterranean sites (Bacarella et al. 1991). Cultivation typically occurred within 80 km of the Mediterranean coast extending onto the slopes of river valleys as well as the interior areas in Spain. Subsequent introductions occurred in 500–600 CE with the conquest of North Africa by Arabs who also brought almonds into southern Spain and Portugal. Two thousand years of continuous cultivation in the Mediterranean basin has concentrated almond plantings into specific regions where well-defined seedling ecotypes have evolved. A tolerance to drought and high susceptibility to soil moisture placed almonds in a mixed culture system with olives, carob, and other desert-adapted crops. Almonds were typically found at higher elevations on well-drained slopes to avoid spring frosts. In these more

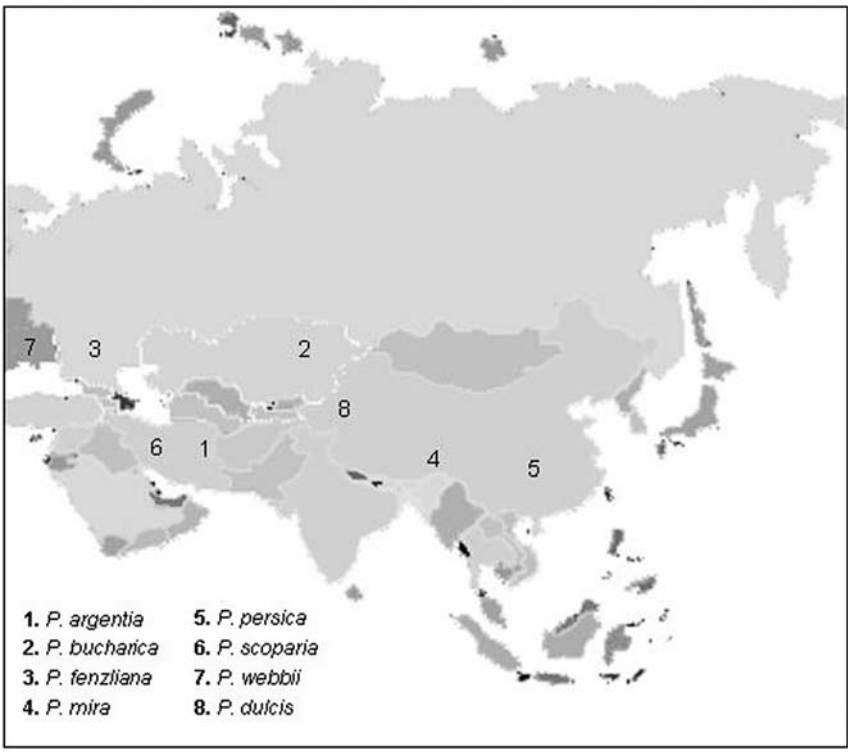

Fig. 1 Map of Asia showing origin of selected almond species

marginal environments, cultural practices evolved which minimized inputs of labor, fertilizers, and use of supplementary water. Locally adapted seedling populations eventually led to a number of local selections adapted to very specific climatic and culture conditions. Selection toward greater local adaptation appears to have been augmented by a more recent introgression of genes from nearby wild almond species. Godini (2000) and Socias i Company (1990) provide evidence for the introgression of self-compatibility and morphological features from *P. webbii* in the development of commercially important cultivars along the northern shore of the Mediterranean Sea.

Both natural- and human-directed*s* selections appear to have occurred both in parallel and in conflict. For example, presence of the bitter kernel gene would be desirable in the wild as it confers resistance to herbivory but would be undesirable for human consumption. Despite its commercial undesirability, most European cultivated almonds are heterozygous for bitterness and many open pollinated seed-derived local land races typically segregate for the bitter kernel trait (Grasselly 1972). The need to graft-over bitter seedlings within these

populations eventually led to selection of local vegetative clones, which subsequently became characteristic of these regions (Bacarella et al. 1991).

The Californian stage initially began as an extension of Mediterranean culture, utilizing a hard-shelled germplasm originally brought from Spain. Later, soft-shell types more compatible for California were introduced from France. High-input orchard practices, however, soon differentiated Californian production from that of Europe and Asia. Important California cultural changes included the movement of almond production from more marginal coastal sites to the very productive Central Valley, the development of new rootstocks and orchard management practices for these highly productive sites, the selection of consistently high-yielding cultivars, and the standardization of markets based upon a relatively few cultivar types. The combination of highly adapted cultivars and rootstocks, favorable soil and climate, abundant water, and effective management has given California growers the highest productivity in the world. Production per hectare continues to show upward trends with yields surpassing 4 MT per hectare presently possible with some cultivar/site combinations.

1.2 Production

The combination of high productivity with extensive plantings has made California the major producer of almonds for commerce with approximately 453,000 MT of nut meats being produced on over 230,000 ha in 2004 (Table 1). Other major almond-producing regions include the European countries bordering the Mediterranean Sea.

Spain, the second leading producer, has a cultivated area of 567,000 ha, producing approximately 26,000 MT in 2004 under primarily low to medium input agriculture. The remaining world production comes from about 20 countries including Italy, Turkey, Greece, and India. Limited almond production extends into the Balkan Peninsula including areas of Bulgaria, Romania, and Hungary. A third area exists in central and southwestern Asia including Syria, Iraq, Israel, Iran, Ukraine, Tajikistan, Uzbekistan, Afghanistan, and Pakistan, extending into western China.

Table 1 Commercial production of almonds in major producing countries (Almond Board of California 2005)

Country	Production (metric ton)
California (USA)	453,000
Spain	26,000
Turkey	14,000
Greece	10,000
Italy	5,000
India	1,000

Many almond species are native to these Asiatic regions where almond growing is often under dryland, low-input culture. Significant almond production also occurs in the southern hemisphere countries having a Mediterranean-type climate including regions in Australia, central Chile, Argentina, and South Africa.

1.3 Uses and Nutritional Composition

The almond kernel is consumed either in the natural state or processed. Because of its good flavor, crunchy texture, and good visual appeal, it has many important food uses (Rosengarten 1984). As an ingredient in many manufactured food products, kernels may be roasted dry or in oil followed by salting with various seasonings (Schirra 1997; Woodroof 1979). The processed kernel is used either blanched or unblanched. Blanching removes the pellicle ('skin') using hot water or steam. Large amounts of kernels are combined with chocolate in confectionery. Almond kernels can be sliced or diced to be used in pastry, ice cream, breakfast cereals, and vegetable mixtures. The kernels are also ground into paste to be used in bakery products and in the production of marzipan. The flavor and texture of almonds can be intensified or moderated through proper selection of cultivar, origin, moisture content, and processing and handling procedures (Kester et al. 1993).

Variation in amygdalin content accounts for some cultivar flavor differences, particularly the distinct amaretto flavor common in certain Mediterranean almonds (Dicenta and García 1993b; Vargas et al. 2001). Californian cultivars had amygdalin contents ranging from 0.33 to 0.84% with only 'Peerless' outside this range at 1.75% (dry weight). In contrast, the Italian cultivars varied from 0.73 to 1.95% with only two cultivars below that range (Schirra 1997). Even higher amygdalin levels will result in bitter almond seeds, which are often blended with sweet.

To obtain the bitter reaction, the substrate amygdalin and a beta-glucosidase enzyme must come into contact through damage to and lysis of the cells. Bitterness results from the hydrolysis of the glucoside amygdalin by a beta-glucosidase enzyme, which produces benzylaldehyde (that confers the 'cherry' or 'amaretto' flavor) and cyanide (which is poisonous) (Kester and Gradziel 1996). Benzaldehyde is also known in the chemical and flavoring industries as 'oil of bitter almond' because of its preponderance in bitter rather than sweet almonds. This trait is typical of the wild almond species where it protects the seed against herbivory.

Almonds are among the most nutrient dense of all tree nuts (Kendall et al. 2003). They are a very good source of essential fatty acids, vitamins, and minerals (Saura-Calixto et al. 1981; 1982) (Table 2). Raw almonds are one of the best plant sources of protein. While certain nut storage proteins can pose

Table 2 Nutrient composition of the almond kernel per 100 g fresh weight of edible portion (Adapted from Socias i Company et al. 2007)

Nutrient	Value
Energy	578 kcal
Protein	21.26 g
Carbohydrate	19.74 g
Fiber, total dietary	11.8 g
Glucose	4.54 g
Starch	0.73 g
Calcium	248 mg
Magnesium	275 mg
Phosphorus	474 mg
Potassium	728 mg
Sodium	1 mg
Folate, total	29 μg
Vitamin E	25.87 mg
Saturated fatty acids	3.88 g
Monounsaturated fatty acids	32.16 g
Polyunsaturated fatty acid	12.21 g

an allergenic health threat to consumers, Sathe et al. (2001) found no significantly elevated risk in a range of cultivated almonds as well as interspecies hybrids. Almonds are also one of the best natural sources of vitamin E (Sabate and Haddad 2001), which is believed to play a role in preventing heart disease, certain kinds of cancer, and cataract formation (Kodad et al. 2006). A single ounce of almonds (approximately 20–25 kernels) contains 37% of the recommended daily value of vitamin E, 21% of magnesium, and 15% of the recommended daily value of phosphorus. Almonds also represent a convenient source of folic acid and fiber (Schirra 1997; Vezvaei and Jackson 1996). Historical uses of sweet and/or bitter almond ointment included the treatment of asthma and pattern baldness; it was also used as a soothing salve for burns.

The almond kernels are also a source of high-quality oil (Abdallah et al. 1998; Garcia-López et al. 1996; Kodad et al. 2005). The oil, which can constitute over 50% of the kernel dry weight, is primarily composed of the more stable oleic acid making it desirable from ancient times to the present for use as a base for various ointments and pharmaceuticals. The high levels of this monounsaturated fat may be partly responsible for the observed association between frequent nut consumption and reduced risk of coronary heart disease (Fulgoni et al. 2002; Lovejoy et al. 2002). Recent evidence has suggested that the incidence of deaths due to coronary heart disease, hypertension, congestive heart failure, and stroke is decreased in people who eat a serving of nuts several times per week (Socias i Company et al. 2007).

Because of their high lipid content (approximately 50–55%), almond kernels are a concentrated energy source (Fraser et al. 2002). The oil is primarily mono-unsaturated, being approximately 65% oleic and 30% linoleic acid, which results in an agreeable supple, buttery flavor, high nutritional value, as well as long-term stability in storage (Fulgoni et al. 2002; Garcia-López et al. 1996;). The hull, which is analogous to the flesh of the closely related peach, contains about 25% sugar and is utilized as a livestock feed. A thorough review of almond nutritional and food quality traits, including opportunities for their genetic manipulation, has recently been compiled by Socias i Company et al. (2007).

2 Botany

While the cultivated almond and its close relatives share basic botanical features and developmental patterns, particularly in the area of reproductive biology, the divergent selection pressures of the wide range of ecological niches occupied have resulted in an extensive variability in final tree and nut form (Felipe and Socias i Company 1992; Niklasson 1989). Within this broad geographical region, extending from the Levant to China, the botanical structure defining commercial quality was a fungible or marketable kernel. High tree productivity, as it increased plant stress, would be a liability in many of the marginal, dryland environments of both ancient and contemporary plantings within these regions. In these harsh environments, primary selection was on tree survival with some level of consistent kernel production regardless of final tree form or physiological pathways. The resulting phenotypic variability offers a wealth of useful traits for cultivar improvement. The divergent development patterns based on unusually similar genomes also offer unique opportunities for the study of the fundamental regulation of plant development.

2.1 Taxonomy

The almond fruit is classified as a drupe with a pubescent skin (exocarp), a fleshy but thin hull (mesocarp), and a distinct hardened shell (endocarp). The hull undergoes limited enlargement during development, later becoming dry and leathery and dehiscing at maturity (Fig. 2). The mature endocarp ranges from hard to soft and papery, depending upon the genotype. Horticulturally, almonds are classified as a 'nut' in which the edible seed (the kernel or 'meat') is the commercial product. The kernel includes an embryo surrounded by the pellicle. Within the *Prunus* genus, the almond is closely related to peach (Felipe 1975). While almond evolved in the xerophytic environment of central and southwest Asia, the peach evolved in the more humid climates of eastern Asia, separated from the almond by the uplifting of the central Asian Massif.

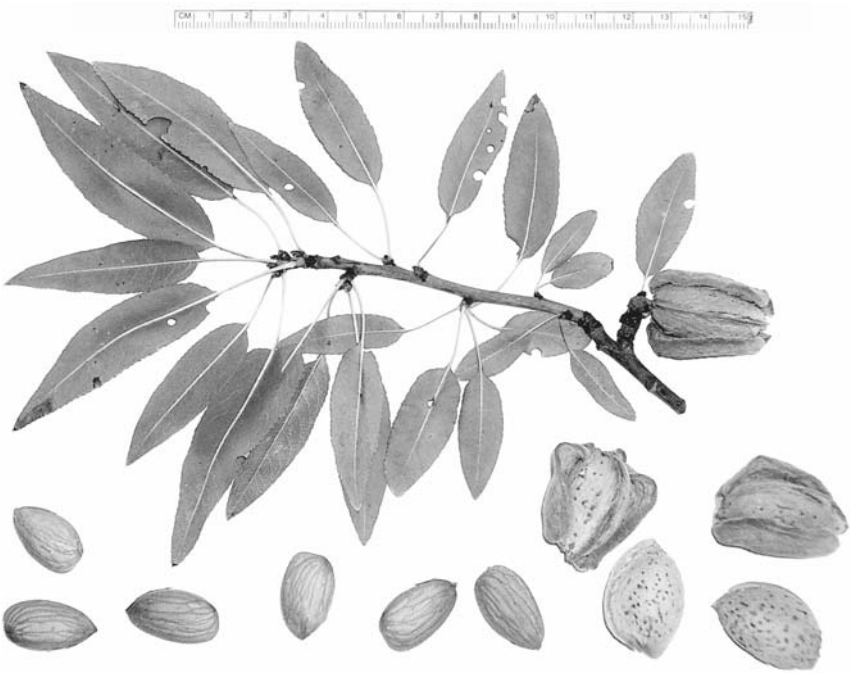

Fig. 2 Cultivated almond shoot showing leaf, fruit, and kernel morphology

Wild populations of almond species representing a wide range of morphological and geographical forms have evolved throughout central and southwestern Asia. Some of the more than 30 species described by botanists may represent subspecies or ecotypes within a broad collection of genotypes adapted to the range of ecological niches in the deserts, steppes, and mountains of central Asia (Grasselly 1972). Browicz (1969) separated almond species into two subgroups: Amygdalus (leaves conduplicate in bud and 20–30 or more stamens) and Dodecandra (leaves convolvulate in bud and fewer than 17 stamens). The most northeasterly group located in western China and Mongolia includes *P. mongolica, P. pedunulata*, and *P. tangutica (P. dehiscens)*, the latter probably in section *Chamaeamygdalus*. The remainder occupies a more or less contiguous area in west central Asia. Almonds in the most northern range include species in section *Chamaeamygdalus* and extend from the Balkan Peninsula to the Altai Mountains. The most southern and xerophytic groups include species in the *Spartiodes* section, which can have leafless slender shoots, and the *Lyciodes* (Dodecandra) section, which are very dwarfed and thorny. Species in the section *Euamygdalus* resembles cultivated almonds and includes many species extending from central Asia to southern Europe (Table 3) as well as the peaches *P. persica, P. mira,* and *P. davidiana*. The chromosome number

Table 3 Botanical relationship of *Prunus* species in subgenus *Amygdalus*

Almond group
Section *Euamygdalus* Spach
Prunus dulcis (Miller) D.A.Webb
P. bucharica Korshinsky
P. communis (L) Archangeli
P. fenzliania Fritsch
P. kuramica Korchinsky
P. orientalis (Mill.), syn. *P. argentea* (Lam)
P. kotschyi (Boissier and Hohenm.(Nab) and Rehd.)
P. korschinskii Hand-Mazz.
P. webbii (Spach) Vieh.
P. zabulica Serifimov
Section *Spartioides* Spach
P. scoparia Spach
P. spartioides Spach
P. arabica Olivier
P. glauca Browicz
Section *Lycioides* Spach
P. spinosissima Franchet
P. turcomanica Lincz.
Section *Chameamygdalus* Spach
P. nana (Stock)
P. ledebouriana Schle.
P. petunnikowi Lits.
P. tangutica Batal.(syn. P. dehiscens) Koehne
Peach group
P. persica (L.) Batsch.
P. mira Koehne
P. davidiana (Carriere) Fransch.

of *P. dulcis (P. amygdalis),* as well as *P. fenzliana, P. nana (P. tenella), P. bucharica, P. kotschyi,* and *P. scoparia*, is $2n = 16$, which is the same as peach *P. persica* (Kester et al. 1991).

2.2 Interspecific Hybrids

While several reports have documented recovery of genes for self-compatibility from related almond species through either natural or controlled crosses (Denisov 1988; Felipe 2000; Gradziel and Kester 1998; Socias i Company and Felipe 1988, 1992), only Rikhter (1969), Grasselly (1972), Denisov, (1988), Kester et al. (1991), and Socias i Company (1990) have previously reported the use of wild species germplasm to create improved almond cultivars. The

historical use of these species and their hybrids as almond rootstocks would facilitate subsequent introgressions. The use of wild species directly as a rootstock for dryland almond has been widely reported, including *P. spartioides* in Iran, *P. bucharica* and *P. fenzliana* in Russia, *P. webbii* in Turkey, and *P. fenzliana, P. bucharica, P. kuramica, P. argentea, P dehiscens*, and *P. kotschyi* at lower incidence in these (Fig. 1) and nearby areas (Gradziel et al. 2001a; Denisov 1988; Grasselly 1972; Rickter 1969; 1972).

Recently, crosses between almond and related species have been readily achieved under controlled conditions (Gradziel and Kester 1998; Gradziel et al. 2001a; Gradziel 2003). While a wide variability in tree and branch architecture results, leaf and nut phenotypes of resultant hybrids are typically intermediate to the parents (Fig. 3). Interspecific crosses between related species (mainly *P. persica* × almond but also *P. webbii* × almond) have been used for almond rootstock breeding in France, USA, Spain, and Yugoslavia (Gradziel et al. 2001b; Denisov 1988; Grasselly 1972; Rickter 1969; 1972; Vlasic 1976). In addition, Browicz and Zohary (1996) and Ladizinsky (1999) have reviewed evidence for a high level of spontaneous interspecific hybridization in the wild between species with overlapping ranges. Surprisingly, the most promising

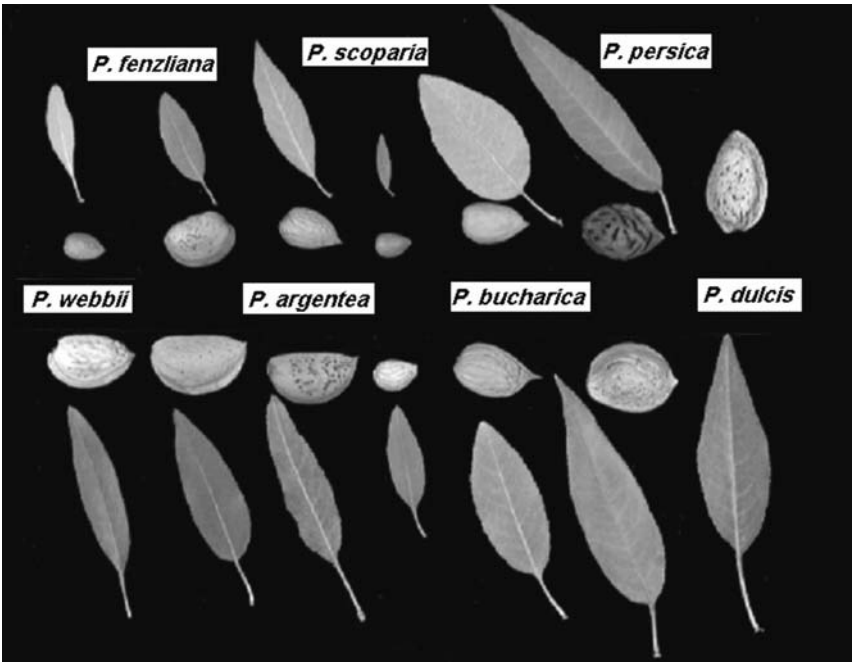

Fig. 3 Leaf and nut morphologies of parent species (*top*) and hybrids with cultivated almond (*bottom*). Typical cultivated almond nut and leaf shown at right

sources of new genes may be the more developmentally distinct peach group including *P. persica, P. mira*, and *P. davidiana*. Rehder (1940; 1967) has placed all of the species examined in this survey in the genus *Prunus*. *P. dulcis* (cultivated almond), *P. persica* (cultivated peach), *P. mira, P. argentia, P. dulcis, P. bucharica,* and *P. fenzliana* are placed in the subgenus *Amygdalus*, section *Euamygdalus*; *P. scoparia* is placed in the section *Spartiodes*; and *P. webbii* placed in the section *Lycioides* (Table 3). However, many Mediterranean and Central Asian researchers prefer the classification of Browicz and Zohary (1996) where *P. persica, P. mira*, and *P. davidiana* are in the genus *Prunus*, while almond and the other almond-like species discussed here were placed in the genus *Amygdalus*. While acknowledging the easy hybridization between almonds and peaches and so the high level of synteny between these genomes, these researchers argue that the divergent evolution of almonds in the harsh climates of central Asia and peach in the temperate to subtropical climates of southeastern China have led to dramatically different growth and development patterns. From an ecological and even taxonomic perspective, these wide divergences suggest their placement in separate genera. Almond and peach thus represent a unique situation in crop plant genetics, where very similar genomes are expressed as very different plant forms. This genome–phenotype disjunction may prove useful for the elucidation and eventually manipulation of the recently recognized epigenetic (i.e., nongenomic) mechanisms, which are now recognized to have profound effects on fundamental plant developmental pathways, and therefore final form and function.

2.3 Reproductive Biology

Almond produces a typical perigynous self-incompatible Prunus flower. Honeybees, foraging for pollen or for nectar secreted at the base of the flower, are important pollinators (Thorp and Roper 1994). Flowers of different cultivars and species may differ in petal size, shape, color, number of stamens, and arrangement and length of anthers relative to stamens. The number of stamens may vary from 20 to about 40 with the usual number being 30–33. The distributions of stamen number within seedling populations from parents of different stamen number indicate quantitative inheritance with a tendency toward dominance of larger numbers. Although the usual pistil number is one, some genotypes such as`Eureka' tend to produce two, which may result in a double fruit. Two flowers can sometimes be produced within the same flower bud with similar results. The structure of the pistil and style also varies. Some styles are straight and elongated, extending above the anthers by petalfall. In other individuals, the stigmas and the anthers are approximately at the same level, a condition associated with increased chances for self-pollination.

Flower differentiation takes place during summer, primarily in August, and floral development continues into the fall and winter (Polito and Micke 1994). Time of flowering is one of the most important adaptive traits of almond as it determines vulnerability to spring frosts. Flowering time is determined by chilling requirements to overcome dormancy and subsequent heat requirements for subsequent bud growth and development. Actual timing of bloom can vary from year to year depending upon the temperature patterns before and during bloom (DiGrandi-Hoffman et al. 1994). In general, the sequence of bloom among different cultivars tends to be fairly constant, but relative bloom time between specific cultivars can sometimes be reversed because of differing requirements for initial chilling or subsequent heat. This relationship is important commercially since it is desirable to have a cultivar flower just before the high-value cultivar in order to maximize its cross-pollination and so yield. For this reason, bloom time is often given relative to a high-value cultivar. In California, 'Nonpareil' is commonly used, whereas in the Mediterranean area, 'Marcona' is frequently the standard.

The cultivated almond as well as most almond species expresses gametophytic self-incompatibility, which discourages self-fertilization, favors cross-pollination, and thus maintains genetic variability within seedling populations. Genetic control of pollen–pistil self-incompatibility is through a single gene (S), which exists in a series of alleles including an allele for self-compatibility. Each diploid genotype carries two alleles of the series. Pollen grains, which have a haploid genome, are unable to fertilize a pistil possessing the same allele. Pollen genotypes with the same S-alleles as the pistil show self-incompatibility as well as cross-incompatibility with other cultivars with the same S-allele. Cross-incompatibility groups (CIGs) have been identified and incompatibility alleles have been assigned to many of them (Boškovic et al. 2003; Channuntapipat et al. 2003; López et al. 2004; Tamura et al. 2000). These groups are important because they guide the selection of cultivar combinations used in orchard planting and provide important gene markers for pedigree studies. CIGs have now been identified for all major California cultivars (Barckley et al. 2006).

Self-fruitfulness refers to the ability of a plant to be fertilized from self-pollen. This competence requires a combination of both self-compatibility and successful self-pollination. Different degrees of self-compatibility exist (Gradziel et al. 2002b). Low pollen tube and ovule growth rates associated with certain genotypes can also decrease the probability of successful fertilization.

Following fertilization, the growth of the fruit, seed, and embryo follows the typical three stages of development in which the pericarp, seed, and nucellus develop during stage I, the endosperm and embryo enlarge during stage II, and the dry weight of the embryo increases during stage III. Time of nut maturity is an important commercial trait. Physiological processes, which accompany almond fruit ripening, include dehiscence of the hull or mesocarp, hull-split, fruit abscission, and dehydration or the loss of moisture in the hull and nut. The entire process of hull and nut maturation and drying may require 2–6 weeks to

complete. Usually maturity is most reliably characterized by the initiation and progress of hull splitting. The dates for the initiation of 5–10% splitting and the completion of splitting are useful criteria when comparison is made to standard cultivars. Moisture stress can accelerate hull splitting, but adequate moisture is required for the hull to ripen properly. If splitting begins prematurely and the nut dries too rapidly, the hull may close tightly on the shell, becoming difficult to remove. In California, the pattern of maturation across the range of almond genotypes extends from early August to late October. Following hull-split, the almond hull and kernel rapidly desiccate to below 7% moisture where mold development is effectively suppressed.

2.4 Tree Characteristics

Trees vary in size, shape, vigor, branching pattern, growth, and bearing habit. Characteristic patterns distinguish cultivars (Brooks and Olmo 1997; Gulcan 1985). These traits affect productivity, training and pruning needs, and adaptability to harvesting operations. Tree size is a relative term that depends not only on the individual genotype but also on orchard age, site (climate, soil), and management (irrigation, fertilization, and pruning). Size is related to precociousness and productivity. Some cultivars such as 'Merced' show reduced growth with age, partly as a consequence of precocious production, while others such as 'Nonpareil' tend to maintain vigor, resulting in larger trees. Size of an individual tree is directly correlated to yield and must be balanced against tree spacing and density to optimize production per hectare. Size of a tree also directly affects management efficiency, depending on the type of cultural system utilized. For mechanized harvest, fewer trees per unit are desirable. If trees are too large, however, they become difficult to shake, prune, and spray. Most cultivated almonds fall within the tree size range of medium to large, depending upon age and site.

Almond, as in all *Prunus*, initiates flower buds laterally on current season growth, which then bloom and fruit the following year (Fig. 2). In general, there are three basic classes of bearing habits: most flower buds on 1-year-old shoots as in 'Ai', most flowers on spurs as in 'Tuono', and mixed as in 'Mission' and 'Nonpareil'. A mixture of both bearing habits is considered advantageous. Shoot bearing habits are associated with precocious production, while spur habit greatly increases the bearing surface. Foliage density is, in turn, determined by the branching habit and the size and distribution of leaves. Foliage density differences can be visually characterized among cultivars. However, leaf size varies with position, with shoot leaves tending to be large and spur leaves small. A classification of growth habits based on variations in primary, secondary, and tertiary shoot development has been described by Gradziel et al. (2002a) and Kester and Gradziel (1990).

2.5 Fruit, Shell, and Kernel Characteristics

Almond fruits of different cultivars vary in size, shape, pubescence, shape and retention of the pistil remnants, and nature of the suture line (Monastra et al. 1982). In 'Drake', the suture line shows a relatively deep depression, while 'Nonpareil' has a relatively smooth line and 'Mission' fruit shows two prominent vertical ridges. The pattern by which 'splitting' occurs in the hull also differs and can be representative of cultivars. Four basic types have been described: ventral split opening on one side ('Peerless'), ventral and dorsal split ('IXL'), four-way split ('California'), and dorsal split ('Jeffries').

The thickness and weight of the mature hull may differ significantly among cultivars. Some hulls such as with 'Mission' are thin and dry and contribute only a small portion of the entire fruit. Others, such as 'Nonpareil', are thick and fleshy and provide a relatively large proportion of the weight. In California, hulls are used for livestock feed and the food value is better with larger hulls. Hull characteristics also affect the relative ease with which nuts are removed from the tree at harvest, the ability of nuts to dry rapidly during harvest, and the ease of hull removal. These processes are more critical with soft-shelled cultivars used in California where worm infestations and concealed damage from wet field conditions can be serious problems.

Shell hardness is associated with the total amount of lignin deposited to the shell during nut development. Shelling proportion (dry weight of kernel/dry weight of in-shell nut) is used to obtain a quantitative measure of shell density and is utilized in commercial activities to calculate kernel yield of different cultivars. Markings on the outer shell are characteristic of individual cultivars as well as different almond species. Within *P. dulcis*, the markings or pores tend to be mostly circular, less frequently elongated, and occasionally a mixture of both. Pores may be large or small, many or few. Other species have smooth and thin shells as with *P. bucharica* or are distinctly grooved or scribed as with *P. kuramica, P. tangutika,* and *P. persica.*

The integrity of the shell, particularly at the suture, is important since poorly sealed shells have kernels exposed and so susceptible to disease and worm damage. The shell consists of an outer and an inner layer separated by channels through which vascular fibers develop. As the hull dehisces and separates from the nut, the outer shell layer may remain attached to the hull and separate from the inner shell layer. The latter type is often associated with high shelling percentages and poor shell seal.

The almond has a large nonendospermic seed having two large cotyledons. Kernel size, shape, and weight are frequently related within cultivars (Arteaga and Socias i Company 2002). Kernel size is often expressed by linear dimensions of length, width, and thickness. These parameters are established during the first growth phase of nut development in the spring and are completed by early summer. Crop density is inversely related to average kernel size. Among kernels

of a given cultivar, a high correlation also exists between dry weight and linear dimensions of length and width. Average kernel weight is an important parameter of yield. Weight increases continuously until maturity. Improper filling may be caused by adverse growing conditions, moisture stress, early ripening, or other environmental and cultural stresses. Shape is a function of relationships among length, width, and thickness. The unique shapes of certain cultivars tend to establish specific marketing categories and uses. Irregularities in width and thickness may change the visual effect significantly. A high correlation was found to exist between width and length among kernels of the same cultivar even when compared in different years and from different locations (Kester and Gradziel 1996). The correlation between thickness and either width or length, however, was much less. As size dimensions decrease, thickness is not necessarily related. Consequently, the relative width to length may appear different for different genotypes otherwise having a similar kernel mass. Shape is usually described from a top view of the wider side of the kernel. Kernels may be round, oval, ovate, oblong, or straight when viewed on one edge, and rounded to various degrees on the other. Thickness (viewed from the edge) may vary from base to tip. Unequal thickness can result in unequal roasting during processing.

3 Breeding

Almonds, either in cultivated orchards or as feral or wild seedlings, have been an important source of food for thousands of years. Within each region, the best wild seedlings were routinely selected for propagation by local farmers, while natural selection continued its unrelenting pressures toward greater adaptation to local environments, including regionally important disease and insect pests. The self-sterile nature of almond insured a continuous exchange and mixing among cultivated and wild germplasm including, in many cases, related species (Grasselly 1972; Socias i Company 2002). Since wild almonds are also harvested for food in these areas, superior genotypes would be identified and propagated. Most modern cultivars in Asia, the Mediterranean area, and more recently in California originated as such time-tested seedlings selections. The subsequent selection over hundreds of years and hundreds of thousands of clonal propagations has also identified improved clonal sources for many of these well-established cultivars. Both genetic (deletions, point mutations, etc.), aneuploidy (see Martínez-Gómez and Gradziel 2003), chromosomal (translocation, see Jáuregui et al. 2001), and epigenetic (gene activation/silencing, etc.) changes would be selected, though because the subsequent selections are vegetatively propagated, the specific nature of inheritance is rarely analyzed.

In the early 1900 s, formal plant breeding programs were established in most major production areas to accelerate this selective process through controlled crosses and related genetic manipulations. While many goals such as total yield

and production efficiency were similar among programs, regional breeding goals often varied due to different environments, disease, and pest problems. At the same time, the globalization of the almond market imposed more stringent limits on acceptable kernel and shell characteristics. Despite inherent obstacles to rapid genetic improvement, including large plant size and the long seed-to-seed generation period of 4 years or more, many commercially successful cultivars have resulted from such controlled crossing programs in the last decades. Examples include the cultivars Ferragnes, Ferraduel, and Ferrastar from France; Butte, Ruby, Sonora, Padre, and Winters from California; and Guara from Spain. Regional almond breeding programs and their primary objectives have been reviewed by Kester et al. (1996).

3.1 Genetic Resources

Cultivated almonds show high levels of genetic variability because their self-sterility makes them obligate outcrossers and possibly due to their interspecies origin. Commercial cultivars within individual production areas, however, often show a limited genetic base due to their origin from only a few founder genotypes selected for their desirable regional value (Felipe and Socias i Company 1992). For example, most commercially important California cultivars originated from crosses between only two parents: 'Nonpareil' and 'Mission' (Bartolozzi et al. 1998; Hauagge et al. 1987; Kester and Gradziel 1996). Greater genetic variability and so increased breeding options for desired traits such as disease resistance are being pursued through the incorporation of breeding material from other regions (Kester and Gradziel 1996; Martínez-Gómez et al. 2003; Socias i Company 1998). Because of the probable interspecies origin of many of these cultivars (Kester et al. 1991; Ladizinsky 1999; Socias i Company 2002), improvement of specific genetic traits may also benefit from the introduction of genes directly from related species. Hybridization between *P. dulcis* and other almond species has often taken place naturally wherever different species come into contact. *P. webbii* grows throughout the Mediterranean region and its range intersects with cultivated almond in Italy Sicily, Spain, and Greece. Hybridization has occurred and introgression evidently results. In the Apulia region of Italia, *P. webbii* has been found to be the source of self-fertility (Godini 2002). The range of almond species is extensive with a wide diversity of traits (Gradziel et al. 2001a; Kester and Gradziel 1996). Controlled crosses of *P. dulcis* with other almond species in sections *Euamygdalus* and *Spartiodes* have been readily carried out (Gradziel et al. 2001a; Gradziel 2003). Hybridization with section *Lycioides* is possible though somewhat more difficult and even more difficult with *Chamaeamygdalus*. Despite their physical and developmental differences, crosses with peach (*P. persica, P. mira*, *and P. davidiana*) can be readily achieved and have proven to be particularly valuable

as rootstocks as well as sources of commercially useful traits (Gradziel et al. 2001a; Gradziel 2003).

3.2 Objectives and Approaches

The goal of cultivar improvement programs is the development of improved cultivars highly adapted to local environments and market demands. Since both market requirements and local adaptation placed considerable limits on the final genetic makeup, most breeding programs pursue the incremental improvement of locally established varieties, typically by the sequential addition of new genetic value (disease resistance, nut quality, maturity time, productivity, etc.). Basic objectives of most almond breeding programs target increased yields, improved quality, and decreased production costs (Socias i Company 1998). These traits have been found to be largely inherited in a quantitative manner (Kester et al. 1977; Spiegel-Roy and Kochba 1981) with a few exceptions such as self-compatibility and kernel bitterness. Heritabilities for important breeding traits have recently been reviewed by Kester et al. (1996), Dicenta et al. (1993a, b), and Socias i Company et al. (2007).

A classical breeding approach toward these goals would involve an initial hybridization between selected parents, followed by introgression of the traits of interest, typically by backcrossing to the parent with the most promising commercial potential. While new genetic engineering techniques offer significant advantages for the discrete addition of new genes to commercially established cultivars, the current dearth of transgenes useful to tree crop breeding limits its present application. Other new biotechnology approaches, particularly gene mapping and gene tagging, offer the promise of greater efficiencies in the areas of gene discovery and gene and introgression (Martínez-Gómez et al. 2006). In addition, the probable interspecies origin of many modern almond cultivars suggests promising opportunities for the manipulation of not only the traditional genetic (i.e., Mendelian) determinants but also the epigenetic controls, which are only recently becoming characterized. Epigenetic modification may have particular value for almonds breeding because epigenetic variability appears to be greatly enhanced with interspecies hybrids (Grant-Downton and Dickinson 2006) and commercially valuable epigenetic variants can be effectively captured in cultivars by the vegetative propagation common in tree crop cultivar dissemination (see Kester et al. 2004).

Epigenetic-like changes (i.e., brought about by an apparent change in gene activity rather than gene DNA sequence) have been documented in clonal differences within cultivars and in a more fully characterized epigenetic disorder known as ‘noninfectious bud failure’. Noninfectious bud failure, which threatens over 50% of California production, is expressed as a deterioration of the clone vitality with increasing age, leading to bud failure in individual trees and

branches. Initial symptoms include the necrosis of the growing point of vegetative buds during the fall. The resulting shoot phenotype, as expressed the following spring, is a failure of terminal and/or subterminal vegetative buds to grow. If the terminal bud fails, 'dieback' results. However, lower and later developing buds may survive providing a 'flush' of new growth at basal and subterminal sites of the shoot. 'Rough-bark' areas sometimes develop in narrow bands on the shoots. New shoots from surviving buds grow vigorously and, when this sequence is repeated in consecutive years, result in an erratic growth pattern, often referred to as 'crazy-top'. Kester et al. (2004) have recently shown that control of this type of epigenetic disorder can be achieved through well-designed certification programs similar to those used to control vegetatively propagated viruses. Such programs have three basic steps: identification of single tree sources which test negatively for the disorder in clonal-source screening trials (see Kester et al. 2004); maintenance and registration of a limited number of trees of the selected clone-source in a foundation orchard; and limited multiplication of registered material to provide certified trees for commercial nurseries (Uyemoto and Scott 1992).

Because epigenetic changes do not respond to traditional breeding methods designed to manipulate classic Mendelian genes, they are generally perceived as undesirable and routinely rouged out using hybridization strategies or for vegetatively propagated crops, clonal selection strategies as described above. However, as both genetic and epigenetic compositions can be captured through clonal propagation, the same methods used to rogue out epigenetic changes can also be utilized to capture desirable epigenetic arrangements. An example would be the widespread practice among nurseries in selecting superior clonal sources of important vegetatively propagated cultivars (Hartman et al. 2002). Epigenetic capture offers unique advantages to breeding programs utilizing wide crosses, since the interspecific hybridization process has been shown to increase the levels of epigenetic variability resulting in novel and transgressive phenotypes (where the trait is expressed at levels beyond the sum of the parents). This breeding approach has recently shown success for peach cultivar improvement where advanced processing peach selections derived from almond–peach interspecific hybridization expressed fruit ripening patterns not evident in either species parent (Gradziel 2003). Regardless of approach, almond breeding objectives typically fall in three general areas: increase yield, improve market quality, and decrease production costs.

3.3 Self-Fruitfulness

Insufficient cross-pollination is frequently the major determinant of commercial yield in self-sterile almond (Asai et al. 1996; Micke 1994). Self-fruitfulness results from the combination of self-compatibility (i.e., self-pollen shows compatible growth to fertilization on pistils of its own flower) and autogamy (i.e., a

flower structure promoting consistent self-pollination). Autogamy appears to be controlled by a number of genes (Kester et al. 1996) affecting flower structure as well as the more dynamic aspects of the flowering process including timing of answer dehiscence (Gradziel and Weinbaum 1999) and pattern of stigma growth relative to maturing anthers (Godini 2002). Although highly autogamous selections have been identified, the genetic manipulation of this trait remains uncertain. Self-compatibility, as with self-incompatibility, is controlled by a major gene (Dicenta and García 1993b), though modifier genes also play important roles (Gradziel et al. 2002b). While many almond species demonstrate some level of self-compatibility, in a cultivated almond background only the self-compatible genes from *P. mira, P. persica,* and *P. webbii* resulted in fruit set above the 30% considered desirable for commercial production (Gradziel 2003a, b). Breeding populations developed from interspecies crosses segregate for self-compatibility in the expected Mendelian ratios for a single gene (Dicenta and García 1993a; Gradziel et al. 2001b; Socias i Company and Felipe 1988, 1992). *P. mira*, the species-cross showing the highest selfing percentages following introgression of the self-compatibility gene, also showed high levels of self-pollination (Gradziel et al. 2001). Long-term efforts to breed self-compatible almonds have been reviewed by Socias i Company (1990).

3.4 Diseases

The most serious foliage diseases of almond include shot hole caused by *Stigmina carpophila* (syn. *Coryneum beijerinkii*), travelure *(Fusicladium amygdali)*, polystigma (*P. occhraceum*), fusiccocum (*Fusicocum amygdali*), and anthracnose (*Gloeosporium amygdalinum* and *Colletotrichum acutatum*). Relative susceptibilities of important cultivars in different countries have been determined and potential sources of resistance have been identified (Kester et al. 1991).

Blossom and twig blight, the major crop-limiting fungal disease worldwide, is caused by (*Monilinia laxa* and *M. cinerea*). These fungi attack the flowers and are most serious in years when rain occurs with bloom. Other fungi, including *Botrytis cinerea*, can also be a serious problem under these conditions. Aflatoxin producing *Aspergillus flavus* infections of the kernel is a major problem, particularly where insect damage is common (Dicenta et al. 2003; Gradziel and Kester 1994; Gradziel et al. 2000; Gradziel and Wang 1994). Although disease control has been possible through fungicides, the need to consider natural resistance becomes more important with the continued loss of agrochemicals.

Almonds can be infected by the same range of viruses as other *Prunus* including the ALAR viruses (ringspot, prune dwarf, line pattern, calico, and apple mosaic) and NEPO viruses (tomato black ring, tomato ring spot, and yellow bud mosaic). Leaf and flower mosaic phenotypes can result from the combination of several viruses. Several complexes of virus-like disorders that

produce 'stem pitting' and 'graft union brown line' are known but not well understood (Uyemoto and Scott 1992). However, many cultivars of almond appear to be immune to the plum pox virus, which remains a serious problem for most stone fruits (Martínez-Gómez et al. 2004).

3.5 Pests

In California, navel orangeworm (*Paramyelois transitella*) and peach tree borer (*Anarsia lineata*) can cause serious damage to nuts at harvest (Rice et al. 1996). This problem is related to the vulnerability of soft, paper-shell, and poorly sealed sutures common to California cultivars, including 'Nonpareil', 'Ne Plus Ultra', and 'Merced' (Gradziel and Martínez-Gómez 2002). Partial control is achieved by integrated pest management, particularly orchard sanitation (IPM Manual Group of U.C. Davis 1985). Resistance through better-sealed shells has been observed in some cultivars including 'Carmel', 'Mission', and 'Butte'. This problem is not serious in the Mediterranean area because of the characteristic well-sealed, very hard, and thick shells of the major cultivars.

Mite species, including pacific spider mite (*Tetranychus pacificus*), two-spotted spider mite (*T. urticae* Koch), European red mite (*Pannonycus ulmi* K), and brown almond mite (*Bryobia rubriculus* Scheuten), can adversely affect production and may be locally important, particularly in conditions of moisture stress. Variation in susceptibility exists among different cultivars.

The almond wasp (*Eurytoma amygdali* End) is an important pest from the Middle East extending into Greece. It attacks the young developing nut. Other significant Mediterranean pests that attack the trunk and branches of trees include *Scolytus amygdali* Guerin and *Capnodis tenebrionis* L. C*apnodis*, a species of borer that attacks the trunk of trees in the Mediterranean basin, particularly trees that are under stress.

3.6 Rootstock Diseases

Crown gall (*Agrobacterium tumefaciens*) can infect the root and crown of nursery trees through previous injuries and then remain with the tree in the orchard where it can cause serious losses (Kester and Grasselly 1987). Peach, almond, and the peach–almond hybrids are susceptible. Oak root or honey fungus (*Armillaria mellea*) is another root fungus of worldwide distribution. Greater tolerance of this problem has been reported in certain plum species but no actual resistance has been described. 'Crown rot', 'wet feet', and 'water-logging' are names given to conditions resulting in deaths of trees associated with excess moisture over a period of time. These conditions have been related to asphyxia and to attacks by various *Phytophthora* species. The symptoms

include dieback at the crown or at smaller roots, depending upon the fungus species, time of year, temperature, and moisture conditions in the soil. Almond, peach, and peach–almond hybrids are generally susceptible, with variation present among species. Plum rootstocks have a higher level of resistance and are the primary rootstocks planted under high soil moisture conditions.

Soil-borne nematodes are problems for almond and peach in many parts of the world (McHenry and Kretsch 1987). Important species affecting almond include root knot (*Meloidygyne incognita* and *M. javanica*), ring nematode (*Criconemoides* spp.), dagger nematode (*Xiphinema* spp.), and lesion nematode (*Pratylenchus* spp.). Dagger nematode is a vector for several viruses, including tomato ringspot virus, which causes 'brown line' in almond, and yellow bud mosaic. Ring nematodes are associated with predisposition of young almond trees to bacterial canker. Root knot nematodes are common in warmer parts of the world with sandy soil. Sources of resistance to *M. incognita* were discovered in certain peach selections including 'Shalil', 'Yunnan', and 'Bokhara' from China and in some almond selections from Israel. Root knot nematodes were later found to have an additional species (*M. javanica*), and a source of resistance was discovered in the wild peach *P. davidiana*.

4 Root Stock Improvement

Almond seedlings have been the traditional almond rootstock used under non-irrigated and well-drained soil conditions. Advantages include easy propagation from seed, excellent compatibility with almond cultivars, deep rooting ability, and high tolerance to drought and calcareous soils. However, almond rootstocks perform poorly on excessively wet soils during active growth. Almond seedling rootstocks are also susceptible to important disease and nematode problems including crown rot (*Phytophthora* spp.), crown gall (*A. tumefaciens*), oak root fungus (*Armillaria* spp.), and root knot, ring, lesion, and dagger nematodes. Because almond rootstocks are very susceptible to fungal diseases and asphyxiation in wet and poorly drained soils, almond cultivars under irrigation are usually planted on three general classes of rootstock: peach, plum, or almond–peach hybrids (Barbera et al. 1994; Rom and Carlson 1985).

4.1 Peach

Almond trees on peach rootstocks grow more vigorously when young, come into bearing somewhat sooner, and tend to survive better than comparable trees on almond rootstocks. The reason for greater tree survival may be a greater tolerance to higher soil water contents, crown rot, and crown gall. Peach is not tolerant of soils that are calcareous, subject to drought, or high in boron. Trees

on peach rootstock are not considered as long-lived as those on almond, but this factor may vary with site conditions, management, and cultivar. 'Lovell' peach seedlings have been the main peach rootstock used in California, though other peach cultivars such as 'Halford' have been substituted with about equal results. With the entry of nematode-resistant or -immune sources, such as 'Nemaguard', a shift has been made to seedlings of nematode-resistant rootstocks in more sandy soils where nematode damage is a problem.

4.2 Plum

Plum species are in a different taxonomic section of *Prunus* than almond and peach and may exhibit incompatibility when used as a rootstock for some almond cultivars (Kester 1970). Almond cultivars may be grafted to certain plum species including *P. cerasifera, P. salicina,* and *P. domestica*. Other plum species rootstocks may survive and grow for long periods but do not provide adequate yield and performance to become a standard commercial rootstock; but they may be potential sources of genes useful for rootstock breeding. Interspecific hybrids between plum and almond, peach, or other plum species have been developed. Important traits possessed by plums include ease of vegetative propagation, resistance to high soil moisture, nematodes, and some diseases such as oak root fungus. The most significant commercial plum rootstocks for almond are the 'Marianna' hybrids—a group of clones arising from a breeding line believed to be *Prunus myrobalan* × *P. hortulana*. Of this group, 'Marianna 2624' is an important rootstock for almond in California for use in finely textured soils with poor drainage and where oak root fungus has occurred. 'Marianna 2624' is also nematode-resistant. Some almond cultivars including the major cultivar 'Nonpareil' can be incompatible on 'Marianna 2624' and related clones, however.

4.3 Almonds × Peach Hybrids

Almonds–peach hybrids generally show strong hybrid vigor and high uniformity. Morphologically, hybrids are intermediate between the parents, and various traits can be exchanged readily between the two species. Particularly useful traits include vigor, nematode resistance, tolerance to replant situations and calcareous soils, and a deep, well-anchored root system. While shoot tip culture can be used to propagate almond–peach hybrid clones, such as 'GF 677' in Europe and 'Hansen' in California, hardwood cuttings provide the most economical nursery clonal propagation method, provided sufficient rooting percentages are obtained. Leafy cuttings (leaf-bud, softwood, or semi-hardwood) under mist or in enclosures can increase the probability of rooting but require higher costs and special facilities.

Micropropagation can increase the range of genotypes propagated, but it also increases the cost of nursery propagation. At the same time, micropropagation has shown promise for the direct rooting of scion material and difficult-to-root rootstock clones and for the rapid increase of new or virus-free cultivars. Explant sources utilized for culture include shoots, leaf petioles, and seed. When endocontamination is a problem, as in long shoot tips and sections of stem pieces, surface-sterilized explants are first placed in a pretreatment medium for 2 or more weeks to allow contaminated material to be identified (Tabachnik and Kester 1977). Scales can also be removed from buds to expose the growing tip, which is then excised and cultured on an appropriate media for elongation and proliferation of lateral buds. The vegetative propagation of almond clones, either as rootstocks or as own-rooted plants, is generally difficult. Shoots of 'Nonpareil' have been established in culture, but rooting and long-term maintenance are difficult.

4.4 Rootstock to Scion Compatibility

The most compatible scion/rootstock combinations are almond–almond. Almond–peach combinations are almost as compatible except that a peach overgrowth generally appears at the union, which can vary by cultivar. No adverse effect has been reported, although some cases of a 'brown line' at the union of 'Milow' almond/'Lovell' peach has been observed. Almond/(peach–almond hybrid) combinations also have smooth unions. Graft combinations of almond–plum and plum hybrids produce varying degrees of incompatibility symptoms (Kester et al. 1965). Graft union abnormalities may occur that cause strong overgrowths or disturbances at the union. This disfunction on 'Marianna 2624' generally occurs only in the bark and not in the sapwood. Symptoms are primarily expressed as disturbance of the normal annual growth patterns, with premature foliage yellowing, and early abscission in late summer and fall. Reduced shoot extension, sparse foliage development, shoot dieback, reduced tree size, excessive spur production, and severe overgrowth tend to follow.

5 Biotechnology

The recent development of powerful new biotechnologies has advanced plant-breeding efforts through the direct incorporation of foreign genes using genetic engineering strategies and through the ability to use a DNA molecule directly as markers for desired traits. While almond cultivars are readily transformed using Agrobacterium-mediated approaches, the regeneration of plantlets from established cultivar cells has proven very difficult. This difficulty is believed to be due to the recalcitrance of cultivar cells to initiate the required organogenesis, presumably because they have lost their juvenility with their advanced clonal age. Molecular markers, however, promise to dramatically increase breeding efficiency as

they offer the opportunity for fast, accurate, and environment-independent evaluation at the seedling stage. In addition, specific markers offer the advantage of codominant expression, good reproducibility, and allow the ability to compare genetic variation among homologous regions of the same or different species (Martínez-Gómez et al. 2003). A detailed review of biotechnology research with almond has recently been provided by Martínez-Gómez et al. (2006).

5.1 Molecular Markers

The most important molecular markers used in almond studies are isozymes, restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs), simple sequence repeats (SSRs), and markers based on unique DNA sequences. Isozymes were one of the first molecular marker evaluations available to almond studies and offered codominant expression and good reproducibility, but were limited by the small number of loci that could be analyzed by conventional staining methods, as well as a low genetic variation at most loci. Nonetheless, it was isozymes studies which first documented extensive genetic variability in almonds overall, as well as the limited genetic base of many almond-breeding programs (Arulsekar et al. 1989; Hauagge et al. 1987; Vezvaei et al. 1995). RFLPs are also codominant but can detect a virtually unlimited number of markers. In almond, RFLPs have been used for discovering linkages between markers, for constructing genetic maps, for cultivar identification, and for the characterization of genetic variability. RAPDs based on PCR amplification of arbitrary primers have been useful for characterizing germplasm variability (Bartolozzi et al. 1998; Martins et al. 2003), but had limited application for cultivar identification and map construction since they are dominant markers with occasional difficulties with repeatability. SSR or microsatellite markers, which are also based on PCR amplification, have proven more useful for genetic relationships (Martínez-Gómez et al. 2003a), cultivar identification (Martínez-Gómez et al. 2003b; Martins et al. 2003), and map construction (Dirlewanger et al. 2004) due to their high polymorphism, codominant inheritance, abundance, and the frequent successful amplification of SSR markers developed in related species (Martínez-Gómez et al. 2006).

5.2 Genetic Linkage Maps

SSR analysis confirmed previous isozymes studies which identified the almond as the most polymorphic species within the major *Prunus* tree crop species (Martínez-Gómez et al. 2006) making it an ideal candidate for map construction. Extensive research, particularly in Europe (see Ballester et al. 1998; Ballester et al. 2001; Corredor et al. 2004; Dirlewanger et al. 2004; Martínez-Gómez et al. 2006), led to the development of a high-density almond map,

which includes 562 markers (361 RFLPs, 185 SSRs, 11 isozymes, and 5 STSs) covering a total distance of 519 cM with an average density of 0.92 cM/marker and largest gap of 7 cM (Dirlewanger et al. 2004). The order of molecular markers observed in the almond map was similar to maps developed with other *Prunus* species suggesting a high level of synteny within the genus (Dirlewanger et al. 2004; Martínez-Gómez et al. 2006). This homology among *Prunus* genomes supports the opportunity for successful interspecific gene introgression as demonstrated by the successful transfer of traits from closely related species to almond (Gradziel et al. 2001a; Martínez-Gómez et al. 2003b). The high level of synteny within the genus also supports the transferability of genetic information developed from linkage maps of other *Prunus* species.

5.3 Trait Mapping and Gene Cloning

The availability of high-density linkage maps has allowed recent successes in establishing the approximate map position of major genes in almond. Important examples include the use of bulk segregant analysis (BSA) to map the self-incompatibility gene (Ballester et al. 1998), as well as a major gene controlling delayed flowering time (Ballester et al. 2001; Grasselly 1978; Socias i Company et al. 1999). Root-knot nematode resistance in an almond–peach hybrid has also recently been reported by Dirlewanger et al. (2004). In addition, the physical mapping of rDNA genes by Corredor et al. (2004) has allowed the establishment of a more precise karyotype for almond.

Cloning of genes expressed during seed development has been reported by García-Mas et al. (1996). Suelves and Puigdomenech (1998) have described the cloning of the mandelonitrile lyase gene responsible for the creation of both cyanide and the amaretto flavor of bitter almonds.

A major effort has been directed toward cloning and characterizing the economically important self-incompatibility gene in almond (Bacarella et al. 1991; Certal et al. 2002). The cDNA encoding almond S-RNase was first cloned by Ushijima et al. (1998). To better understand the nature of the self-incompatibility gene, Ushijima et al. (2001) later cloned and characterized the cDNA encoding mutated S-RNase from the almond cultivar 'Jeffries', which has a disfunctional S-allele haplotype in both pistil and pollen.

5.4 Marker-Assisted Selection

PCR-based markers of almond self-incompatibility S-alleles have been successfully used to identify different self-incompatibility genotypes (Barckley et al. 2006; Channuntapipat et al. 2003; Tamura et al. 2000). Similar results were obtained by Boškovic et al. (2003) who identified major almond cultivar stylar

S-RNase by electrophoresis in vertical polyacrylamide gels. PCR-based markers of almond self-incompatibility S-alleles have been employed to facilitate the integration of self-compatible S-alleles from related species (Gradziel et al. 2001a). Screening efficiency and flexibility has been greatly increased with the development of successful multiplex PCR techniques by Sánchez-Pérez et al. (2004). Using advanced cloning strategies, Ushijima et al. (2003) have recently described the structural and transcriptional analysis of a pollen-expressed F-box gene with haplotype-specific polymorphism strongly associated with self-incompatibility.

Molecular markers are currently being employed to elucidate the genetic basis of plant processes controlled by multiple genes. For example, Campalans et al. (2001) have described a differential expression technique based on cDNA-AFLP (amplified restriction fragment polymorphism derived technique for RNA fingerprinting) to characterize genes involved in drought tolerance in almond. Results identified increased drought tolerance in specific genes associated with leaf function.

Despite these recent advances in the application of the newer biotechnologies, almond, as well as other tree crops, lags behind the progress typically observed for annual crops. This is, in large part, the consequence of the inherent difficulties in doing genetic studies on such large-sized and long generation-time plants (Martínez-Gómez et al. 2003). However, these inherent obstacles to traditional breeding make the opportunities with the new technologies much more revolutionary when applied to tree crops. When fully integrated with the array of breeding methods developed to capitalize on the inherent advantages of tree crops, such as the capability to capture desirable genetic/epigenetic arrangements through vegetative propagation, breeding potential could be expected to surpass that for seed-propagated annual crops. Almond is currently well positioned to be a leader in this effort.

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Breeding Apple (*Malus* × *Domestica* Borkh)

S. Pereira-Lorenzo, A.M. Ramos-Cabrer, and M. Fischer

1 Introduction

The apple tree is a hybrid originating from a combination of wild species (*Malus sieversii* is supposed to be the main contributor). Growers at first selected the best specimens by seedlings, but when grafting was discovered as a mean of vegetative propagation, improvement in fruit quality became faster. Apple is cultivated in most of the temperate regions due to the fruit's quality, its easiness to propagate, and its natural aptitude to bear. Apples are considered a healthy fruit, as the saying goes 'an apple a day keeps the doctor away'. An apple tree can reach up to 10 m height above its own roots, having a globose canopy and the longevity between 60 and 100 years. Depending on the rootstock and the age of the tree, the roots can occupy between 2 and 104 m^2, although most frequently they range between 10 and 30 m^2 (Atkinson 1980).

1.1 Reproductive Biology

The apple tree is a monoecious species with hermaphroditic flowers. Three to six flowers in cymes (the first flower is the most advanced) appear in mixed buds (Dennis 1986, 2003). It produces rose epigynous flowers, sometimes white, with five sepals, petals, and pistils and up to 20 stamens. The development of a multicarpellate inferior ovary (forming the core) and the accessory tissue after fecundation becomes in the fruit known as pome (Ryugo 1988).

Apples are self-incompatible though some cultivars are partially self-compatible (Lespinasse 1992). Most of the apple cultivars are diploid ($2n = 34$ chromosomes) and some of the main cultivars are triploid ($2n = 3x = 51$), e.g., 'Boskoop', 'Kaiser

S. Pereira-Lorenzo
Escola Politécnica Superior, Departamento Producción Vegetal, Universidad de Santiago de Compostela, Campus de Lugo, 27002
e-mail: santiago.pereira.lorenzo@usc.es

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Wilhelm', 'Gravensteiner', 'Jonagold', and mutants, 'Kanadarenette' and others. Triploids are not suitable as pollinators. Mostly wild species are diploid, and a few are triploid and tetraploid. Pereira-Lorenzo et al. (2007) and Ramos-Cabrer et al. (2007) found that 29% of the local cultivars in northern Spain were triploids, producing an average of 15% heavier apples.

Parthenocarpic apple cultivars exist but they are not relevant in commercial production (Dennis 1986). As most cultivars are self-unfruitful, cross-pollination, mainly by insects, is necessary. Knowledge of possible combinations is needed for the best success of apple production (Table 1), since only 10–30% of the flowers develop into fruit.

Self-incompatibility in apple is of gametophytic type and is controlled by a single multiallelic locus named the S-locus (Broothaerts et al. 2004). Pollen

Table 1 Cross-pollination between new apple cultivars (adapted from Fischer 2000)

Pollinator Mother cultivar	Pia	Piflora	Pikant	Pilot	Pingo	Pinova	Pirol	Piros	Reanda	Rebella	Reka	Relinda
Pia	–	+		+	+	+	+	+	+	+	+	
Piflora	+	–		+	+	+	+	+	+	+	+	
Pikant		+	–	+	+	+		+	+	+	+	+
Pikkolo			+	+		+		+				
Pilot	+	+	+	–		+			+	+	+	
Pingo	+	**o**		+	–	+	+		+	+		
Pinova	+	+	+	+		–	+	+	+	+	+	
Pirol	+	+			+	+	–	+	**o**	+		
Piros			+			+		–	+		+	
Reanda		+	+	+		+	**o**	+	–	+	+	+
Rebella	+	+	+	+	+	+	+	+		–	+	+
Regine	+	+	+	+	+	+	+	+	+	+	+	+
Reglindis		**o**	+			+			+	+	+	+
Reka			+	+		+		+	+		–	+
Releika				+	+	+	+	+	+	+	+	
Relinda									+			–
Remo	+		+	+		+	+	+	+		+	+
Rene	+		+								+	+
Renora	+	+	+	+	+	+	+		+	+	+	+
Resi						+	**o**	+		+		+
Retina	+	+	**O**				**o**	+	+	+	+	+
Rewena	+	+	+	+		+		+	+	+	+	+
Elstar			+	+		+		+		+	+	+
Golden Delicious	+	**o**	+	+	+	+	+	+	+	+	+	+
Idared	+	+	+	+	+	+	+		+	+		
Jonagold	+	**o**	**O**	**o**			+			+	+	**o**
Prima			**O**				**o**				+	
Shampion			+	+		+						

Table 1 (continued))

Remo	Renorn	Resi	Retina	Rewena	Elstar	Golden Delicious	Idared	James Grieve	Jonagold	Prima	Shampion
	+	+	+	+	+	+	+	+	−		
		+	+			o					
+	+	+		+	+	+	+	+	−	+	+
+	+					+	+	+	−		+
+				+	+	+	+	+	−		o
		+	+		+		+				
+		+	+	+	+	+	+	+	−		+
		+	+		−	+	+	+			
+	+		+			+	+	+	−	o	+
+			+	+		+	+	+	−	+	
+	+	+	+	+		+	+	+	−		
+	+	+	+	+	+	+	+	+	−		
+			+				+	+	−	+	
+	+	−	+	+		+	+	+	−	+	
+	+	+	+	+			+	+			
+			+	+			+	+		+	
−	+	+	+	+	+	+	+	+	−	+	
+			+	+			+	+			
+	−	+	+	+			+	+	−	+	
+		−	+	+			+	+	−	+	
+			−	+		+	+	+	−	+	
+			+	−		+	+	+	−	+	
+			+	+	−	+		+	−	+	−
+		+	+	+	+	−	+	+	−		
+		+		+		+	−	+			+
			o	−	o	−	+	+	−		−
+			+					+		−	
					o	o	+	+	−		−

tubes elongate through the styles. As they grow, they are attacked by cytotoxic proteins. Expression of specific inhibitors avoids a lethal attack. Style toxic proteins are the product of the S-gene (S-RNases). Pollen tube growth is inhibited when the pollen shares the same S-allele with the pistil on which the pollen germinates. Eighteen different S-alleles have been differentiated; only three of them are the most frequent (*S2, S3*, and *S9*).

1.2 Main Species

Apple, pear, plum, and peach trees belong to the Rosaceae family. Apple and pear, as other genera, have been classified inside Maloideae family because they produce pome type fruits (Bretaudeau and Faure 1991; Janick et al. 1996).

Scientific nomenclature for apples has changed since Linnaeus denominated *Pyrus malus*. Other denominations in the past have been *M. communis*, *M. sylvestris*, *M. pumila*, and *M. domestica* (Ryugo 1988; Harris et al. 2002). The domesticated apple is the result of an interspecific hybridization named *Malus* × *domestica* Borkh (Janick et al. 1996). This name has been substituted to the previous *M. pumila* (Forsline et al. 2003). The cultivated apple is a functional diploid ($2n = 34$) (Ryugo 1988) although it is frequently present as triploids (Pereira-Lorenzo et al. 2007).

The number of species in the genus *Malus* is uncertain and still under controversy. Robinson et al. (2001) explained that the number of species in genus *Malus* depends upon the rank given to several taxa, species being subspecies and putative hybrids, and the nomenclature of the taxa is complex. Harris et al. (2002) pointed out about 55 species (between 8 and 79 have been recognized) according to the classification of Phipps et al. (1990). Zhou (1999) classified 30–35 species. Only 17 are recorded in the USDA, NRCS (2006) Plants Database (www.plants.usda.gov) (Table 2) (Fig. 1).

Way et al. (1990a, b) gave details of 33 main species. Forsline et al. (2003) referred to 27 primary species, 5 secondary ones, and 11 *Malus* species hybrids. Janick et al. (1996) counted 37 species and 16 secondary ones (*Malus* species hybrids). Way et al. (1990a, b) and Janick et al. (1996) agreed in nine species in series Pumilae in which they also included *M. domestica* and *M. sieversii*. Forsline et al. (2003), however, denominated that series as Sieversinae, including *M. sieversii* but not *M. domestica* as it has now been considered a natural hybrid, *M. x domestica*. The classification of 27 primary apple species according to Forsline et al. (2003) is included in Table 2, with indications of the origin and use when these are known. A total of 22 of 27 species (82%) are from Asia (11 located mainly in China), 4 in North America, 2 in Europe, and 1 in Japan. Six species are used for fruit—*M. sieversii*, *M. sylvestris, M. angustifolia, M. ioensis, M. coronaria*, and *M. hupehensis*. Five out of 27 are recognized as ornamental and 12 as possible rootstocks.

1.3 Climatic and Environmental Requirements

The apple tree adapts well to different climates. Apple is cultivated from northern Europe down to the tropics where two crops can be obtained at high altitudes. It has been introduced in South America, South-Africa, New Zealand, and Australia. Most of the old cultivars require a long rest period, but new selections with less requirements allow them to be cultivated in subtropical areas. Petropoulou (1985) classified apple cultivars attending to chilling requirements in six classes and related a shorter rest period with lower growth (Table 3). Some cultivars are very resistant to low temperatures (−35°C). Some were selected for very short seasons, 3 months from blooming, while others require up to 6 months.

Table 2 Species in *Malus* genus (adapted from Forsline et al. 2003; USDA, NRCS 2006)

Sections	Series	Primary species	Commom name	Origin	Uses
Malus Langenf.	*Sieversinae* Langenf.	*M. sieversii* (Lodeb.) Roem.		Asia	Rootstock, fruit
Malus Langenf.	*Sieversinae* Langenf.	M. orientalis	Caucasian apple	Asia	
Malus Langenf.	*Sieversinae* Langenf.	*M. sylvestris* (L.) Mill.	European crabapple	Europe	Fruit, roostock
Baccatus Jiang	*Baccatae* (Rehd.) Rehd.	*M. baccata* (L.) Borkh.	Siberian crabapple	Asia	Rootstock, ornamental
Baccatus Jiang	*Hupehenses* Langenf.	*M. hupehensis* (Pampan.) Rehd.	Chinese crab apple, tea crab apple	China, Taiwan	Rootstock, ornamental, fruit
Baccatus Jiang	*Hupehenses* Langenf.	*M. halliana* (Anon.) Koehne	Hall crab apple	China	Rootstock, ornamental
Baccatus Jiang	*Sikkimenses* Jing	*M. sikkimensis* (Wenzig) Koehne		Asia	Rootstock
Sorbomalus Zabel.	*Sieboldiane* (Rehd.)	*M. sieboldii* (Regel) Rehd.	Toringa crab apple	Asia	Rootstock, ornamental
Sorbomalus Zabel.	*Kansuenses* (Rehd.) Rehd.	*M. kansuensis* (Batal.) Schneid.		China	
Sorbomalus Zabel.	*Kansuenses* (Rehd.) Rehd.	*M. transitoria* (Batal.) Schneid.		China	Rootstock
Sorbomalus Zabel.	*Kansuenses* (Rehd.) Rehd.	*M. toringoides* (Rehd.) Hughes	Cutleaf crab apple	China	Rootstock
Sorbomalus Zabel.	*Kansuenses* (Rehd.) Rehd.	*M. komarovii* (Sarg.) Rehd.		China, North Korea	

Table 2 (continued)

Sections	Series	Primary species	Commom name	Origin	Uses
Sorbomalus Zabel.	*Kansuenses* (Rehd.) Rehd.	*M. xiaojinensis* Cheng et Jiang		China	
Sorbomalus Zabel.	*Kansuenses* (Rehd.) Rehd.	*M. fusca* (Raf.) Schneid.	Oregon crabapple	Northern America	
Sorbomalus Zabel.	*Yunnanenses* Rehd.	*M. yunnanensis* (French) Schneid.		Asia	Rootstock, ornamental
Sorbomalus Zabel.	*Yunnanenses* Rehd.	*M. prattii* (Hemsl.) Schneid.		China	
Sorbomalus Zabel.	*Yunnanenses* Rehd.	*M. honanensis* Rehd.		China	
Sorbomalus Zabel.	*Yunnanenses* Rehd.	*M. ombrophilla* Hand.-Mazz.		China	
Sorbomalus Zabel.	*Florentinae* Rehd.	*M. florentina* (Zuccagni) Schneid. (= *M. crataegifolia* (Savi) Koehne)	Florentine crab apple, hawthorn-leaf crab apple	Europe	
Chloromeles (Decne.) Rehd.		*M. ioensis* (Wood.) Brit.	Prairie crabapple, western crab apple	Northern America	Ornamental, fruit
Chloromeles (Decne.) Rehd.		*M. coronaria* (L.) Mill.	sweet crabapple	Northern America	Ornamental, fruit
Chloromeles (Decne.) Rehd.		*M. angustifolia* (Ait.) Michx.	Southern crabapple	North America	Fruit, ornamental
Docyniopsis Schneid.		*M. doumeri* (Bois.) Chev.		Asia	Rootstock
Docyniopsis Schneid.		*M. melliana* (Hand.-Mazz.) Rehd.		China	

Table 2 (continued)

Sections	Series	Primary species	Commom name	Origin	Uses
Docyniopsis Schneid.		*M. tschonoskii* (Maxim.) Schneid.	Pillar apple	Japan	
Docyniopsis Schneid.		*M. laosensis* Chev.		Asia	Rootstock
Eriolobus (D.C.) Schneid.		*M. trilobata* (Poiret) Schneid.		Asia, Europa	
Cultivated species *Malus* and secondary species including hybrids					
M. × a*rnoldiana* (Rehd.) Sarg. (*baccata* × *floribunda*)					
M. × *atrosanguinea* (Spaeth) Schneid. (*halliana* × *sieboldii*)					
M. × *domestica* Borkh.			Cultivated apple		Fruit, rootstock
M. × *hartwigii* Koehne (*halliana* × *baccata*)					
M. × *micromalus* Mak. (*baccata* × *spectabilis*)					
M. pumila Miller					
M. × *purpurea* (Barbier) Rehd. (*neidzwetzkyana* × *atrosanguinea*)					
M. × *soulardii* (Bailey) Brit. (*ioensis* × *domestica*)					
M. × *sublobata* (Dipp.) Rehd. (*prunifolia* × *sieboldii*)					
M. × *asiatica* Nakai					
M. × *dawsoniana* Rehd. (*fusca* × *domestica*)					
M. floribunda Siebold					
M. × *magdeburgensis* Schoch. (*spectabilis* × *domestica*)					
M. × *platycarpa* Rehd. (*coronaria* × *domestica*)					
M. prunifolia (Willd.) Borkh.					
M. × *robusta* (Carr.) Rehd. (*baccata* × *prunifolia*)					
M. spectabilis (Ait.) Borkh.					
M. zumi (Mats.) Rehd. (*mandshurica* × *sieboldii*)					

Fig. 1 Species in *Malus* genus (adapted from Forsline et al. 2003; USDA, NRCS 2006) (With credits to USDA, ARS, Plant Genetic Resources Unit, Geneva) (*See* Color Insert)

Fig. 1 (continued)

Table 3 Chilling requirements of cultivars and rootstocks apple (adapted from Petropoulou 1985)

Cultivar	Chilling requirements	Days to blooming
Rome Beauty	2700–3100	201
Ingrid Marie	2300–2700	152
Keswick Codlin	2300–2700	136
Antonouka	1900–2300	129
Kidd's Orange Red	1900–2300	115
Early Victoria	1450–1900	106
Cox	1000–1450	99
Winter Banana	1000–1450	98
Falstaff	300–1000	77
Starkspur Golden Delicious	300–1000	77
Greensleeves	300–1000	74
Rootstocks		
M16	2300–2700	139
M25	1800–2300	103
M7	1350–1800	65
M27	950–1350	55
M9	950–1350	44

2 History

The origin of the cultivated apple, *Malus x domestica*, is the genepool of *Malus sieversii* in Middle Asia. Vavilov located the center of origin for *Malus communis* (*M. sieversii*) in Turkistan, Central Asia (Vavilov 1951). The use of molecular markers could confirm that the wild apple located in Central Asia could be the major maternal contributor to the domesticated apple (Harris et al. 2002). Wild fruits were selected and propagated by indigenous populations before 6500 B.C. Cultivation and domestication moved westward along the Silk Road and possibly along a second northern way across Central Russia. Introgression of *Malus orientalis* and *M. sylvestris* var. praecox and *M. sylvestris* var. sylvestris was reduced in *Malus x domestica*. The first mention of cultivated apples in ancient Greece dates from the 9th century B.C. Later on, apples were introduced into the Mediterranean regions and Central Europe by the Romans. Columela wrote about grafting and the most preferred apple cultivars in the year 42 A.D. Greeks and Romans spread the culture across Europe. In East Asia, crossings between *M. sieversii* and *M. baccata* developed the hybridogenic species *Malus x asiatica*, which has been used as local fruit crop since ancient times (Tian shan) (Büttner et al. 2000). In the middle ages, apple culture was promoted greatly around monasteries. By the end of the 12th century, some famous cultivars were known, such as 'Pearmain' and 'Costard' (Morgan and Richards 1993). In the 16th century, dwarf rootstocks were recommended to graft selected cultivars (Tubbs 1973). By the beginning of the 20th century, the main objective of

breeding was to transfer the high-quality traits of the fruit along with resistance to three economically important apple diseases: fire blight, scab, and powdery mildew. A review can be found further on.

2.1 Cider History

Orton (1995) explained how the first apple beverage could have been made with crab apples (wild apples). Hebrews called cider 'Shêkar', the Greeks 'Sikera' (a drink obtained by cooking apples with fermented juice), and a beverage, 'Phitarra', was obtained by boiling pieces of apples in water with honey in the Basque country (www.applejournal.com/fr05.htm). By the end of the 4th century, the Latin word 'Sicera' was introduced, becoming Cider in English, Sidre and Cidre in French, and Sidra in Spanish. In France and Spain, apple trees for cider production were planted abundantly from the 10th and 11th century on (Boré and Fleckinger 1997; Rivas 2004). In the 15th century, fruit growing specialists recommended the use of sour-sweet apples to improve taste and the addition of a few acid apples to avoid blackening. With the spreading of phylloxera among the vineyards, cider began to replace wine.

Cultivars for cider production were essential in the development of the cider industry. The first apple description for cider production (65 cultivars) was published in France in 1589 (Boré and Fleckinger 1997). The first selections for cider production also began in France in 1883 with a detailed study for each region. During 1949 and 1970, more than 1000 cultivars were collected and identified, 70 of them being recommended for cider production. Since 1953, five cultivars have been selected for juice production in France ('Judor', 'Jurella', 'Judeline', 'Judaine', and 'Juliana') and one for cider ('Cidor'). In Germany, some of the multiresistant Re-cultivars® have been recommended for processing since 1990 ('Remo', 'Rewena', 'Relinda', and 'Rene') (Fischer et al. 2001a).

Cider apple production dropped considerably between 1968 (2 millions t) and 1990 (650,000 t) (Boré and Fleckinger 1997). Although new plantations are being established, cider production is based mainly on traditional orchards with high vigor and scattered apple trees, over 10 million in 1990. A similar situation can be found in Spain where regular plantations for cider production only account for about 8000 ha in the northern regions (MAPA 1990).

3 Socioeconomic Importance

3.1 Area and Production

Apples are cultivated mainly in temperate zones, and they adapt very well to different climates. The cultivated area in 2004 was 51.6 million ha with a total production of 61.6 million t (Table 4). The main area of apple production is

Table 4 Apple area (ha) and production (t) in 2004

Country	Area (ha)	Production (t)	Country	Area (ha)	Production (t)
Albania	2300	120,000	Australia	30,000	484,096
Belarus	68,000	200,000	New Zealand	11,000	500,000
Belgium	8272	323,800	Oceania	41,000	984,096
Czech Republic	12,700	280,781			
France	58,180	2,216,940			
Germany	70,000	1,592,000	Morocco	26,100	393,140
Greece	15,500	288,000	Egypt	29,000	490,000
Hungary	36,000	680,000	Algeria	30,000	1,262,444
Italy	61,469	2,069,243	South Africa	31,000	762,558
Moldova, Republic of	70,000	338,000	Tunisia	32,000	121,000
Netherlands	10,000	436,000	Africa	148,100	3,029,142
Poland	160,000	2,500,000			
Portugal	21,600	287,600	Israel	6000	125,000
Romania	120,235	1,097,837	Armenia	8000	300,000
Russian Federation	386,000	16,000	Lebanon	9400	140,000
Serbia and Montenegro	27,000	183,571	Turkmenistan	12,000	40,000
Slovenia	3293	230,000	Kyrgyzstan	25,000	123,000
Spain	40,000	603,000	Korea, Republic of	26,000	350,000
Switzerland	5190	230,000	Georgia	28,000	60,000
Turkey	108,900	2,300,000	Tajikistan	40,000	93,000
Ukraine	150,000	850,000	Kazakhstan	41,000	140,000
UK	9000	125,000	Japan	41,300	881,100
USSR		2,030,000	Pakistan	48,000	380,000
Others	89,439	446,450	Syrian Arab Republic	48,000	215,000
Europe	1,533,078	19,444,222	Azerbaijan, Republic of	50,000	220,000
			Korea, Dem People's Rep	71,000	660,000
Peru	9900	146,083	Uzbekistan	94,000	500,000
Canada	20,813	370,338	Iran, Islamic Rep of	150,000	2,400,000
Brazil	32981	977,863	India	250,000	1,470,000
Chile	39,000	1,250,000	China	2,100,550	22,163,000
Argentina	40,000	56,000	Others	10,148	105,636
Mexico	62,000	503,000	Asia	3,058,398	30,365,736
USA	162,500	4571,440	World	**5,160,190**	**61,823,546**
Others	12,420	125,626			
America	379,614	8,000,350			

Source: www.fao.org

located in Asia, a nucleus that accounts for nearly double in terms of area and production in comparison with Europe. The main producers are China, Poland, Turkey, France, USA, and Algeria.

3.2 Market Uses

Apples are produced mainly for the fresh market (Way and McLellan 1989). In the USA, apples are processed into five basic products, viz., juice, canned puree, canned slices, dried apples, and frozen slices. Apple juice and canned sauce are the dominant products (one-half and one-third, respectively). Apples are also processed into vinegar, jelly, apple butter, mincemeat, and fresh slices. Small quantities are also made into apple wine, apple essence, baked whole apples, apple rings, and apple nectar. All these products represent between 44% and 46% of the apple production in the USA (Way and McLellan 1989). Another important product is cider, mainly in France, the UK, and Spain, although it is gaining popularity in the USA. Smock and Neubert (1950) consider that the most important product prepared from apples is pure fermented apple juice or cider, except in the USA and Canada.

Clarified apple juice is the main product and its preparation involves pressing, clarification treatment, filtration, and packaging (Bump 1989). Natural apple juice comes from the press and the addition of ascorbic acid or heating makes it flocculate and form unstable compounds. Pulpy (crushed) apple juice has a light color and a high pulp content. To produce pulpy apple juice, washed apples are coarsely grinned and the mash processed in a pulper with a fine screen. The pulped juice is passed through a vacuum chamber for deaeration to minimize oxidation and preserve its light color. Frozen apple juice concentrate production is based on a concentrate of 43° Brix to 70–74° Brix that is reconstituted in clarified or natural types by adding water. Apple juice and concentrates are used as base for blended fruit juices and drinks.

In the USA, 'apple wine' is distinguished from cider by its higher content of alcohol due to the adding of sugar during fermentation or by adding alcohol after fermentation or both (Smock and Neubert 1950). 'Apple brandy', a distilled cider product, can be used directly for consumption or for fortifying apple wine. The meaning of the term cider can vary depending upon the region of the world (Downing 1989). In England, it is known as 'fermented juice', 'hard cider' in the USA, 'cidre' in France, 'sidre' in Italy, 'sidra' in Spain, and 'Apfelwein' or 'Apfelmost' in Germany and Switzerland. There are several types of cider depending upon the preparation method (Smock and Neubert 1950; Downing 1989). 'Sparkling sweet cider' is produced by fermenting apple juice just enough to give it some effervescence and it contains less than 1% alcohol by volume. Fermentation and further steps are carried out in a closed system to retain the natural carbon dioxide that forms. 'Sparkling cider' also

retains gas produced during fermentation, but it has a low sugar and high alcohol content (3.5%). 'Sweet cider' is a noneffervescent cider produced by partial fermentation of apple juice in an open tank or by adding sugar to a completely fermented juice. 'Dry cider' is a completely fermented apple juice, commonly called 'hard cider', with an alcohol content of 6–7%. 'Carbonated cider' refers to any cider charged with commercial carbon dioxide to produce effervescence. 'Champagne-type cider' is produced in a similar way to champagne, effervescence being produced in the final product by a secondary fermentation of the dry cider in bottles. Sugar and champagne yeast are added before bottling.

Sugar is responsible for the softness in apple juice, whereas acid (normally measured as malic acid) gives it the tartness. Tannins support astringency, referring to the bite, the body, or the pungency (Downing 1989). Levels of sugar and acid are normally measured by chemical tests, while astringency is judged best by taste. Juice is preferred to make the body of soft cider not too sweet or too heavy (Downing 1989). Astringency is less significant than a correct sugar–acid ratio and the juice should not have more than 0.1% tannin. Downing (1989) pointed out that juice used for fully fermented and sparkling cider should be high in sugar, of moderate acidity, and fairly astringent.

4 Genetic Resources

4.1 Centers of Origin

In 1930, Vavilov suggested that Turkistan was the area where *M. sieversii* and *M. domestica* could have originated (Robinson et al. 2001). These wild species produce apples quite similar to domestic ones. This area offers a great variety in apples; therefore many authors agree that Central Asia is the center of origin of *M. domestica* (Janick et al. 1996). Zhou (1999) referred China as the origin place since about 80% of all species of the genus can be found in this country. Büttner et al. (2000) suggested that some *Malus* species with large fruits developed between Middle Asia to Central Europe. They consider that out of that genepool in Middle Asia, *M. sieversii* contributed the most to the origin of *M. x domestica*. The 'Silk Road' could have brought about introgressions of *M. orientalis* and *M. sylvestris* var. praecox from Caucasia and southeastern Russia. According to these authors, the indigenous species in Central Europe, *M. sylvestris* var. sylvestris, were not involved in the domestication of the apple. However, Boré and Fleckinger (1997) and Luby (2003) pointed out that hybridization could have contributed to diversify local apple cultivars. Janick et al. (1996) and Forte et al. (2002) consider that *M. sieversii* could have hybridized with other species such as *M. orientalis, M. sylvestris, M. baccata, M. mandshurica*, and *M. prunifolia*. With the support of the nuclear ribosomal internal transcribed

spacer (ITS), Harris et al. (2002) explained that the Central Asian wild apple and the domesticated apple can be grouped with *M. asiatica*, *M. orientalis*, *M. niedzwetzkyana*, and *M. prunifolia*. Apple selections could have been introduced directly from wild species in Western Europe and later on, hybridizations could have been important in bringing about new cultivars with specific characteristics (Harris et al. 2002).

4.2 Germplasm Banks Worldwide

An extensive review on European *Malus* germplasm has been made available (IPGRI 1996). More than 30,000 accessions are conserved *ex situ*. Most of those accessions were characterized and evaluated using IBPGR (1982) and UPOV (1974) descriptors. However, these efforts were not enough to compare the complete variability found in Europe. A minimum number of data (Passport data) was collected in European *Malus* database (Maggioni et al. 1997) without success.

In the USA, a total of 4179 accessions are maintained in repositories, of which 1456 corresponded to *Malus x domestica* (www.ars-grin.gov). The exact number of accessions is still unknown.

One of the most important European *Malus* gene banks is located in Germany (Dresden-Pillnitz) with more than 300 accessions of *Malus* species and hybrids, and nearly 1000 apple cultivars from around the world (Fischer and Fischer 2000; Fischer et al. 2003).

In Spain, the main resources are located in Asturias, Galicia, Navarra, País Vasco (Basque country), and Zaragoza (Dapena 1996; Itoiz and Royo 2003; Pereira-Lorenzo et al. 2003; Pereira-Lorenzo et al. 2007). Research programs are focusing on the development of apple cultivars for dessert and cider production.

High costs and damage risks from pests and diseases or the environment encouraged the development of cryopreserved, dormant apple buds for cultivars (Forsline et al. 1998; Forsline 2000). On populations, Volk et al. (2005) evaluated the minimum number of seedlings needed to capture more than 90% of the genetic diversity of the original populations and stated that a total of 35 trees within each population should be used as parents in crosses in order to obtain seeds for long-term *ex situ* conservation of *M. sieversii*.

5 Breeding Objectives and Tools

5.1 Cultivars

Until the mid 20th century, most apple cultivars were selected from seedlings (Janick et al. 1996). Apple diversity is very high due to polymorphism (Pereira-

Lorenzo et al. 2003, 2007), but commercial types depend on a reduced number of cultivars. Noiton and Alspach (1996) determined that 64% of 439 selections had their origin in among five cultivars: 'McIntosh' (101 cultivars), 'Golden Delicious' (87 cultivars), 'Jonathan' (74 cultivars), 'Cox's Orange Pippin' (59 cultivars), and 'Red Delicious' (56 cultivars). Among them, 96 cultivars had two or more as parents. Other cultivars used frequently in crosses were 'James Grieve', 'Rome Beauty', and 'Wealthy'.

Estimations have shown that in the last 5 years, 43% of the registered cultivars in France were mutations from commercial cultivars in use at the time and six of them cannot be differentiated clearly from the originals (Le Lezec et al. 1996).

This reduced number of cultivars used in breeding programs can be explained by the lack of information of the germplasm banks, which reduces their possible use (Noiton and Alspach 1996). The main problem when using a reduced number of cultivars is the inbreeding among future generations, in comparison with other fruit trees as peach, raspberry, or chestnut. Nowadays, new approaches can be afforded to increase genetic variability in commercial releases such as collecting seedling from the supposed original species *M. sieversii* (Forsline and Aldwinckle 2004) or using old cultivars (Lateur and Populer 1996a, b). But in breeding, inbreeding problems are not yet visible in seeding populations due to the elevated heterozygocity of the genus *Malus*.

The first cultivar obtained by crossing was attributed to Thomas Andrew Knight (1759–1838). Another method to obtain new cultivars consisted in the selection of mutations and chimeras (Janick et al. 1996); these develop shoots with a stable variation when they are propagated vegetatively. The crossing of two parents is now, as it always has been, the main method in apple breeding (combination breeding).

Genetic transformation has the advantage that it maintains cultivar identity since (Brown and Maloney 2005). Although progress is being made, there are problems with the field-testing of transgenic apples as quality traits are too complex to be improved by this biotechnology. The expression of transformed genes is still uncertain and needs more methodical and practical research. On the other hand, the general acceptance of genetic modified organism (GMOs) is not very good and it requires more information for the public. Maybe it would be better if it were possible to transfer species-owned genes instead of foreign genes, like fire blight resistance (Krens et al. 2004).

Currently, the main characteristics of cultivated apples are (1) size over 100 g or 70 mm as a minimum for the market; (2) colors: yellow, green, red, bicolor, and brown in susceptible apples to russeting; (3) acidity: sweet apples when malic acid is lower than 4.5 g/L and bitter when it is over that limit; (4) tannins: sharp apples are those with more than 2 g/L of tannic acid; (5) sweetness: most of the cultivars contain between 12° and 18° Brix; (6) harvesting period from August to December; and (7) resistance to diseases and abiotic stress.

The eating quality is difficult to measure objectively (Hampson et al. 2000). Contribution of crispness accounts for about 90% of the variation in texture liking. Juiciness, aroma, sweetness, and sourness change their relative importance from year to year. They account for about 60% of variation in flavor liking. Sweetness and sourness are better predictors of liking than analytical measurements of soluble solids and titratable acidity. Formal sensory evaluation is a reliable way for screening breeding selections (Hampson et al. 2000). Some researchers have found poor correlation between soluble solids (% SS), titratable acidity (TA), and firmness with sensory perceptions of sweetness, sourness, and texture (Bourne 1979a, b, c; Watada et al. 1981).

The main cultivars used for cider are differentiated on the basis of their acidity and tannin levels. Four groups of apples can be classified considering acidity and tannin contents (Downing 1989; Lea 1990): bittersweet apples contain more than 0.2% (w/v) tannins and less than 0.45% (w/v) acidity (calculated as malic acid). Sharp apples have less than 0.2% (w/v) tannins and more than 0.45% (w/v) acidity. A subgroup of this classification, bittersharps, has the same range of acidity but a tannin content over 0.2% (w/v). Sweet apples have less than 0.2% (w/v) tannins and 0.45% (w/v) acid.

Different types of apples should be mixed to obtain a good cider (Downing 1989). Low-acid cultivars for the basic juice and high acid levels add tartness to the cider. Aromatic cultivars as 'Cox's Orange' add flavor and bouquet to a cider. Astringent apples can improve body and flavor. As a rule, no more than 10% of astringent cider should be added to an acidic cider and no more than 20% should be added to any blend. Apples should be mature and free from starch. Blending with fermented stock is preferred since the fermentation of fresh juice cannot always be predicted. Cider apples have a higher tannin and sugar content than culinary apples but are lower in acid (Downing 1989). Dessert and culinary apples lose more body and flavor due to fermentation than cider apples.

The ideal cider apple is slightly riper than the fresh market one (Downing 1989). As apples mature, the starch turns into sugar, increasing sweetness and flavor. Unripe apples produce juice with a 'starchy' or 'green apple' flavor. Acidity and astringency also decrease after harvest, both with a pronounced effect on the flavor of the juice.

If we compare commercial cultivars' characteristics (Iglesias et al. 2000) with some of the most frequently used cider apple cultivars in Spain (Table 5), we can say that the acidity of various groups, such as 'Elstar' and 'Reinetas,' is equivalent to some cider cultivars, such as 'Raxao'. Cultivars producing high levels of tannins are rarer, such as 'Teórica' or 'Collaos'.

Some special characteristics can be important in the use of specific cultivars, as (1) sensibility to *russeting,* which produces a brown aspect that is specific in some cultivars such as 'Reineta Gris de Canada', 'Boskoop'; (2) growing habit, spur types, and weeping; (3) late blooming; (4) high cold hardiness; (5) resistance

Table 5 Main characteristics from main commercial cultivars and local ones

Cultivar	Blooming	Harvest	Color	Caliber (mm)	Brix	Tanins	Acidity	Firmness (kg)	Origin
Commercial cultivar Gala Group*	5 Apr	10–25 Aug	Bicolor	73–82	12–14		2.6–4.9	7–9	New Zealand
Elstar Group*	10 Apr	10–30 Aug	Bicolor	74–78	13–15		7.7–9.9	6–7	Golden Delicious × Ingrid Marie
Delicious Group*	5 Apr	1–15 Sept	Red	75–91	11–15		2.5–3.4	7–8	Seedling, USA
Golden Group*	20 Apr	15–25 Sept	Yellow-Green	69–90	13–17		3.6–9.0	6–10	Goden Reineta × Grime Golden, USA
Reinetas Group*	20 Apr	5–15 Sept	Yellow-Brown	74–85	12–18		11–13	8–10	Ancient cultivars, origin unknown
Jonagold Group*	10 Apr	1–15 Sept	Bicolor	80–92	14–17		5–6	5–8	Delicious × Jonathan
Braeburn Group*	1 Apr	25 Oct to 5 Nov	Bicolor	74–84	12–14		5–7	8–9	Seedling, New Zealand
Granny Smith Group*	14 Apr	1–25 Nov	Green	78–90	12–13		9	8	Seedling from French Crab, Australia
Fuji Group*	10 Apr	5–25 Nov	Bicolor	70–84	13–17		3.1–4.2	7–9	Seedling from Ralls Janet × Red Delicious, Japan
Local cultivars									
Blanquina	9 May	32 Oct	Yellow-Green	50–70	12	0.9	4.4	11	Spain
Collaos			Red			4.9	2.1		Spain
Cristalina	13 May	23 Oct	Red	56–65	12	0.9	5.7	11–12	Spain
De la Riega			Bicolor			1.4	5.2		
Marialena	5–11 May	22 Sept	Bicolor	53–59	13	1.4	4.8	8–9	Spain
Raxao	20 May	23 Oct	Bicolor	56–71	12	1.5	7.7	10–12	Spain
Teórica	12 May	22 Oct	Red	47–58	14	4.0	14.5	11–13	Spain
Mingán		25 Oct	Bicolor	60–66	12–15	2	3	10–13	Spain
Tres en Cunca	4 Apr	20 Sept	Yellow	53–72	13	1.2	7.2	10	Spain

*Data adapted from Iglesias et al. (2000)

to pests and diseases; and (6) local cultivars that need less treatment in comparison with commercial ones and are desired in their area.

5.1.1 Genes and Effects

Main characteristic genes have been localized in different cultivars and are used in breeding programs (Table 6). Genes related with ethylene biosynthesis (ACS, ACO, and ACC) regulate conservation and fruit softening. Albinism (*al* gene), pale green lethal seedlings (*l* gene), and color due to anthocyanin genes, as well as greasy skin (*Gr* gene), have been studied by several researchers. Genes affecting petals have been found controlling apetaly (*ape*) or double petals (*Pd*). Tobutt (1994) related apetaly with the ability to produce parthenocarpy fruits. Some genes have been found affecting fruit quality, such as aroma (*Ar*), malic acid (*Ma*), or bitter pit (*Bp-1* and *Bp-2*). Sensibility to russeting is attributed to the *Ru* gene (Alston and Watkins 1975). Genes related to chilling requirement (*Chr*) and early budbreak (*Ebb*) have been discussed by Decourtye and Brian (1967) and Lawson et al. (1995), respectively. Studies in growth regulation have provided deep knowledge in apple dwarfing (genes *st-1* and *st-2* for sturdy dwarf; genes *dw-1, dw-3*, and *dw-4* for early dwarf; and *cr* for crinkle dwarf), regrowth promoter (*G*), Gibberellin gene, Knotted 1-like homoeobox expressed during growth and development, gene *MdPIP1* controlling fruit expansion and in plants under osmotic stress, and *DAD1* as inhibitor of programmed cell death. Genes related with apple fertility are *MADS-box* genes associated with the development of floral meristems and organ identity, *MDH1* (apple homoeobox gene) involved in the control of plant fertility, pollen lethal (*P-1, P-2, P-3, P-4*, and *P-5*), and pollen incompatibility (S-alleles).

Also, different pest- and disease-resisting genes have been localized (Table 6), such as genes for curling aphids resistance (*Dysaphis devecta* Wlk.) (genes *Sd-1* to *Sd-3* and the precursor *Pr-Sd*), for WAA resistance (*Eriosoma lanigerum*) (*Er-1*), for yellow mottle (*ym-1, ym-2, ym-3*), for hypersensitivity to *D. plantaginea* (*Sm-h*), for fire blight resistance (Alston and Briggs 1970), for *Glomerella cingulata* susceptibility (*Gb*), for *Gymnosporangium* resistance (*Gy-a* and *Gy-b*), for *Phyllosticta solitaria* susceptibility (*Ps-1* and *Ps-2*), for *Phytophthora cactorum* resistance (*Pc*), for *P. leucotricha* resistance (*Pl-1*, *Pl-2, Pl-w*, and *Pl-d*) by and the precursor *Pr-Pl-1*, and for scab resistance (*Venturia inaequalis*) (*Va, Vb, Vbj, Vf, Vfn, Vm, Vr, Vr2*).

Quantitative traits loci have been studied for branching habit, vegetative bud break, reproductive bud break, bloom time, and root suckering, using molecular markers (Lawson et al. 1995), in combination with random amplified polymorphic DNAs (RAPDs) to study juvenile tree growth (Conner et al. 1998). Quantitative trait loci (QTLs) for stem diameter, plant height increment, leaf size, bloom traits, juvenile phase, and fruit characteristics have been evaluated by Liebhard et al. (2003), fruit quality by King et al. (2000), scab resistance by Calenge et al. (2004), and powdery mildew resistance by Stankiewicz-Kosyl et al. (2005).

Table 6 Apple genes for different characteristics

Gene denomination or abbreviation	Gene effect	References
1-aminocyclopropane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxydase (ACO).	Ethylene biosynthesis during ripenning	Dong et al. (1991); Dong et al. (1992); Castiglione et al. (1999); Harada et al. (2000); Costa et al. (2005)
1-aminocyclopropane-1-carboxylic acid (ACC) synthase, 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase)		
1-aminocyclopropane-1-carboxylic acid synthase (ACS)	Fruit softening	Oraguzie et al. (2004)
E-1, *E-2*	Early ethene production	Batlle (1993)
Polygalacturonase (PG)	Hydrolyze pectins that softens the fruit	Dong et al. (1998)
Al	Albinism	Crane and Lawrence (1933)
l	Pale green lethal seedlings	Klein et al. (1961)
Anthocyanin biosynthesis genes	Skin color	Kim et al. (2003)
UDP glucose-flavonoid 3-O-glucosyltransferase (pUFGluT)	Anthocyanin expression in apple skin	Kondo et al. (2002)
Rf	Anthocyanin in fruit skin	Wilcox and Angelo (1936); Wakasa et al. (2003).
Rt	Anthocyanin in all tissues	Sampson and Cameron (1965)
Gr	Greasy skin	Alston and Watkins (1975)
Ru	Russetted fruit skin	Alston and Watkins (1975)
Ape	Apetaly	Tobutt (1994)
Atc	Atrophied corolla	Decourtye (1967)
Ca-a, *Ca-b*	Deciduous calyx	Henning (1947)
Pd	Double petals	Sampson and Cameron (1965)
Ar	Aromatic fruit flavor	Alston and Watkins (1975)
Ma	Malic acid	Nybom (1959)
Bp-1, *Bp-2*	Bitter pit resistance	Korban and Swiader (1984)
Yfl	Yellow/cream flesh	Crane and Lawrence (1933)
Chr	Chilling requirement	Decourtye and Brian (1967)
Ebb	Early budbreak	Lawson et al. (1995)
Co	Columnar habit	Lapins and Watkins (1973)
Sp-1, *Sp-2*, *Sp-3*	Spur type habit	Decourtye and Lantin (1969)
dw-2	Compact habit	Decourtye (1967)
W	Weeping habit	Sampson and Cameron (1965)

Table 6 (continued)

Gene denomination or abbreviation	Gene effect	References
Tb	Terminal bearing	Lawson et al. (1995)
st-1, *st-2*	Sturdy dwarf	Alston (1976)
dw-1, *dw-3*, *dw-4*	Early dwarf	Alston (1976)
Cr	Crinkle dwarf	Alston (1976)
G	Regrowth promoter	Alston (1976)
Gibberellin 20-oxidase gene	Hormone	Kusaba et al. (2000)
Knotted 1-like homoeobox	Expressed during growth and development	Watillon et al. (1997)
MdPIP1	Fruit expansion and in plants under osmotic stress	Hu et al. (2003)
DAD1	Inhibitor of programmed cell death	Dong et al. (1998)
MADS-box genes	Development of floral meristems and organ identity	Sung and An (1997); Sung et al. (1999); Sung et al. (2000); Yao et al. (1999); Van der Linden et al. (2002)
MDH1, apple homoeobox gene	Involved in control of plant fertility	Watillon et al. (1997)
P-1, *P-2*, *P-3*, *P-4*, *P-5*	Pollen lethal	Heilborn (1935)
S	Pollen incompatibility	Kobel et al. (1939)
S-alleles	Pollen incompatibility	Boškovi· and Tobutt (1999); Broothaerts et al. (1995); Broothaerts et al. (2003); Kitahara and Matsumoto (2002a, b); Kobel et al (1939); Matityahu et al. (2005)
Sd-1 to *Sd-3*, precursor *Pr-Sd*	Curling aphids resistance	Alston and Briggs (1968, 1977); Roche et al. (1997)
Er-1	Woolly apple aphid (WAA) resistance	Knight et al. (1962); Sandanayaka et al. (2003)
ym-1, *ym-2*, *ym-3*	Yellow mottle	Sadamori et al. (1964)
Sm-h	*Dysaphis plantaginea* hypersensitivity	Alston and Briggs (1970)
Gb	*Glomerella cingulata* susceptibility	Thompson and Taylor (1971)
Gy-a and *Gy-b*	*Gymnosporangium* resistance	Aldwinckle et al. (1977)
Ps-1 and *Ps-2*	*Phyllosticta solitaria* susceptibility	Mowry and Dayton (1964)
Pc	*Phytophthora cactorum* resistance	Alston (1970)

Table 6 (continued)

Gene denomination or abbreviation	Gene effect	References
Pl-1, *Pl-2*, *Pl-w*, and *Pl-d*; precursor *Pr-Pl-1*	*Podosphaera leucotricha* resistance	Knight and Alston (1968); Dunemann et al. (1999); Markussen et al. (1995); Alston et al. (2000); Batlle and Alston (1996); Batlle (1993); Dayton (1977); Korban and Dayton (1983)
Va, *Vb*, *Vbj*, *Vf*, *Vfn*, *Vm*, *Vr*, *Vr2*		Dayton and Williams (1968); Barbieri et al. (2003); Patocchi et al. (1999a); Patocchi et al. (1999b);

5.1.2 Local Cultivars

Normally, apple production, as with other crops, focuses on regular plantations established with a few highly productive genotypes of extraordinary quality. However, great quantities of apples are produced in small orchards, generally established with local cultivars that form reservoirs of the main origins of variability. These cultivars have been selected locally and rusticity is normally one of their main values. But they also satisfy the acceptance of local consumers, having more flavor, likely due to their aptitude to be cultivated with less sprays in order to achieve a more ecological production.

If we take into account some of the main characteristics that define an apple cultivar (Table 5), local cultivars present similar characteristics to those broadly spread. Possibly, the old fashion look of the local varieties is their most outstanding characteristic. Standardization and globalization in marketing apples have hardly reduced the number of varieties cultivated. Brown and Maloney (2005) have pointed out the importance of name recognition in marketing apples. In Spain, most of the local cultivars have nearly disappeared from commercial orchards. However, the situation can change in the future with the revalorization of local products and Denominations of Origin, as it has happened previously with winery grapes. Local cultivars contribute greatly in cider production, no doubt due to the lower price of cider apples, but this has also tended to reduce interest in their breeding. Presently, the knowledge in local apple cultivars is increasing, a situation which can serve to diversify the apple market (Pereira-Lorenzo et al. 2003; Díaz-Hernández et al. 2003; Itoiz and Royo 2003; Pereira-Lorenzo et al. 2007).

Some of the resistant local varieties could be used in breeding in order to transfer polygenic resistance, a very important fact considering the first breakdown of the monogenic scab resistance of *M. floribunda* (Kemp et al. 2004; Fischer et al. 2001b).

5.1.3 Growth Habit

Until now, vigor and growth habit were controlled by dwarfing rootstocks and growth regulators with the aim to establish high-density orchards. A new approach focuses on the genes involved in tree architecture, such as columnar, *Co*, which does not allow the growth of lateral branches and the fruit appear in spurs over the main axe (Tobutt 1985, 1994; Quinlan and Tobutt, 1990). Although some new cultivars with this gene have been released ('Maypole', 'Tuscan', 'Trajano', 'Telamon'), it has been recognized that still a lot of work must be done in order to achieve a similar gustative quality among the present cultivars.

Four fruiting types have been proposed by Lespinasse (1992) based on the vegetative growth and fruiting habit:

1. Columnar. An axe is covered with spurs. It is controlled by a dominant gene and was previously discovered in 'Wijcik McIntosh'. It hardly needs pruning and tends to bear biannually.
2. Spur. It is characterized by short shoots in the scaffold limbs. Trees tend to be upright and numerous spurs appear close to the trunk. It tends to hold a biannual production.
3. Spindle. It is presented by ('standard') 'Golden Delicious'. Varieties tend to be spreading with wide crotches and frequent branching. They bear on spurs and shoots that are generally 1–3 years old. The fruiting zone tends to move away from the trunk to the outer sides of the tree (IBPGR 1982).
4. Tip bearer, characterized by 'Granny Smith'. Varieties tend to have upright main scaffold limbs with narrow crotches and frequent branching (IBPGR 1982). They bear a large part of the crop upon the ends of the previous year's shoots. This kind of cultivar has a shorter production time and a more regular bearing pattern than type 1 or 2 (Lauri and Costes 2004).

5.1.4 Stylar Incompatibility and Molecular Markers

Apple varieties exhibit a self-incompatibility mechanism, preventing fertilization following self-pollination (reviewed by De Nettancourt 2001). Pollination studies based on microscopic evaluation of pollen-tube growth through the pistil allowed to discriminate 11 different S-alleles in apples (*S1–S11*) Kobel et al (1939) and 26 cultivars were classified. Using IEF and NEPHGE followed by RNase activity staining, Bošković and Tobutt (1999) identified the gene product for *S1–S11* and they added 14 more S-alleles (numbered *S12–S25*). To resolve the discrepancies in S-allele assignment, Broothaerts (2003) reexamined the identity of S-alleles known from domestic apple cultivars, designing allele-specific primer pairs to selectively amplify a single S-allele per reaction. Highly similar S-alleles that were coamplified with the same primer pair were discriminated through their distinct restrictive digestion pattern. In most cases,

Broothaerts results (2003) coincided with those obtained through phenotypic and S-RNase analysis.

5.2 Rootstocks

Rootstocks have been used at least from Roman times as they were used to graft selected cultivars onto seedlings (Tubbs 1973). The first reference in the UK of 'Paradise' as a dwarf apple tree was in 1597. A 1629 reference describes how this tree was used as rootstock to develop small trees. Dwarfing rootstocks known as 'Paradise' or 'Doucin' in 18th century Europe were a mixture (Ferree and Carlson 1987). Fourteen different types were mentioned in 1870.

Extensive reviews on apple rootstocks can be found in Ferree and Carlson (1987), Masseron (1989), Webster and Wertheim (2003), Wertheim (1998), and Brown and Maloney (2005). Rootstock breeding has focused on size control (dwarfing), tolerance to low temperatures (hardiness), tolerance to pathogens and pests, and on adaptability to different soil conditions (Brown and Maloney 2005). Dwarfing was very effective respecting the seedlings (14 m high) to 30–40% of 'M.27' (Masseron 1989).

There is no easy explanation about the size control by the rootstock. Some hypotheses include graft union anatomy (Soumelidou et al. 1994), the ABA:IAA ratio in dwarf rootstocks (Kamboj et al. 1999), and hydraulic conductivity and dwarfing (Atkinson et al. 2003). Atkinson et al. (2003) found a lower hydraulic conductivity in dwarfing rootstocks compared with semivigorous rootstocks. These observations were consistent with lower xylem-to-phloem ratios and changes in xylem vessel anatomy in dwarf rootstocks, which might explain their influence in shoot behavior when used on grafted plants. Soumelidou et al. (1994) suggested that the failure of auxin in cross-graft union with dwarf rootstocks reduces rootstock xylem production, with poor water and mineral supply to the scion. Kamboj et al. (1998) measured a higher ratio of ABA:IAA in dwarf rootstocks.

The main diseases that affect rootstocks are crown and root rot (*Phytophthora* spp.), fire blight (*Erwinia amylovora* Burrill Winslow et al.), and canker (*Nectria*). WAA (*E. lanigerum* Hausmann) has been considered the main pest. Other problems affecting rootstocks are burknots, genes *bu-1, bu-2* (Decourtye 1967), and root suckering, *Rs*, (Lawson et al. 1995).

Although seedlings from wild or cultivated apples were and are the main origin of rootstocks, clonal rootstock 'MI.793' (hybrid between Northen Spy and M.2) appeared in 1989 in nurseries (Masseron 1989).

The most frequently used rootstocks in the world were selected in the UK during the 20th century. 'M.2', 'M.7', and 'M.9' belong to the serial East Malling (EM) obtained between 1912 and 1913 by Hatton and now denominated 'M1' to 'M16' (Masseron 1989). They were selected from populations

used in different countries. 'M.9' is the most used rootstock in Europe and comes from the population 'Paradis Jaune' from Metz, and some clonal and sanitary selections were recently obtained: (1) United Kingdom, 'M9 EMLA'; (2) The Netherlands, 'M9 NAKB'; and (3) France, 'PAJAM 1' ('Lancep') and 'PAJAM 2' ('Cepiland').

'M.106' and 'M.111' belong to the serial Malling Merton with 15 types, from 'M.101' to 'M.115', resistant to *E. lanigerum* and medium to strong vigor (Masseron 1989). They were selected in 1952 from crosses between 'Northen Spy' and various selections of the serial EM. 'M.106' are some of the most interesting rootstocks for cider production (Díaz-Hernández et al. 2003) because they provide the minimum vigor avoiding trellis. Díaz-Hernández et al. (2003) compared the two most interesting rootstocks, 'M106' and 'M111', with some important cider apple cultivars in northern Spain. As reported previously (Masseron 1989), 'M.106' showed less vigor, although not significantly different and induced a considerably higher productivity than 'M.111' for 'Reineta Encarnada' and 'Teórica' (Díaz-Hernández et al. 2003). 'M.25', 'M.26', and 'M.27' belong to the serial Malling (M). They were selected in 1960 and are not resistant to *E. lanigerum*.

Breeding programs initiating in 1953 in the USA have been reviewed by Brown and Maloney (2005). Several selections of the Cornell Geneva series (CG) are fire blight resistant and are under study. One of the most well-known American rootstocks is 'Michigan Apple Clone 9', 'MAC 9', which preformed poorly in hot dry soils (Webster and Wertheim 2003).

Al-Hinai and Roper (2004) established a trial to check if different rootstocks influence the growth and quality of 'Gala' fruits. They used four rootstocks, 'M.26', 'Ottawa 3', 'M.9 Pajam 1', and 'Vineland (V)-605'. In conclusion, rootstocks had no effect on fruit growth, final size, or yield. Apple fruit size was influenced by the crop load. When Marini et al. (2002) adjusted the effect of apple rootstocks on the weight of 'Gala' fruits for crop load, they found differences between rootstocks but agreed that longer period of study would be necessary.

Rootstock has effect on gene expression patterns and, therefore, over grafted scions. Jensen et al. (2003) discussed the different influence of 'M.7' rootstocks (with reduced susceptibility to fire blight) and 'M.9 NAKB T337' ('M.9 T337') rootstocks (highly susceptible to fire blight). They found differences in the expression of a number of photosynthesis-related, transcription/translation-related, cell division related genes and stress-related gene expression; therefore, expressed genes might influence the tree stature, stress tolerance, photosynthetic activity, and fire blight resistance.

New challenges on apple dwarfing rootstocks are being considered as seen in selections from Russia ('B.146' and 'B.491'), Sweden ('BM 427'), USA ('G.65' and other CG- and G-rootstocks), Japan ('JM.1', 'JM.5', and 'J.M.8'), Czech Republic ('J-TE-G'), UK ('M.20'), Poland ('P.22', 'P.59', 'P.61', 'P.66'), Canada ('V.3'), Germany ('Supporter' 1 to 4), and Romania ('Voinesti 2') (Webster and Wertheim 2003).

5.3 Molecular Markers

Traditional methods for identification and classification of cultivars are based on morphological and agronomical characters, being the only methods that are legally recognized at present (Bailey 1983; REGLAMENTO (CE) N° 2100/94 DEL CONSEJO DE 27 de julio de 1994, D.O.C.O. 1.9.94). As with morphology, molecular markers were first used to focus on cultivar identification due to the relevance in breeding in order to have sharp differentiations between cultivars that have not been disturbed by environmental influence. The first studies in isoenzymes for clonal identification were done by Chyi and Weeden (1984), Menendez et al. (1986a, b), Weeden and Lamb (1985), and Manganaris (1989).

Nowadays, multiple molecular markers exist as isoenzymes, restriction fragment length polymorphisms (RFLP), RAPD, microsatellites, amplified fragment length polymorphism (AFLP), SCAR, or ISSR that allow differentiating varieties (Karp and Edwards 1998). The molecular markers are biomolecules that can be related with a genetic characteristic. There are two general types of molecular markers: proteins and DNA. The first markers, developed at the end of the 1970s, were based on the identification of proteins and isoenzymes. Isoenzymes constitute a system of multiple molecular forms of enzymes in which heterogeneity is partly due to genetic factors and partly to posttranslational modifications (Moss 1982).

Identification techniques of proteins and isozymes are based on electrophoresis analysis in starch gel (Smithies 1955; Torres 1989) and in the visualization of enzymatic products by histochemical methods (Hunter and Markert 1957). This technique is considered to be a magnificent tool to evaluate genetic resources (Karp et al. 1997), and it continues to be one of the most frequently used markers among investigations in genetic diversity of forest trees (Wagner et al. 2004). Its main limitation is the relatively low level of polymorphism detected in comparison to molecular markers based on DNA.

RFLP is a technique based on hybridization of complementary strands. RAPD , AFLP, and SSR (simple sequence repeats) or microsatellites were developed using polymerase chain reaction (PCR) technique that amplifies specific areas of DNA.

SSR markers offer greater advantages in respect to another molecular markers, because they are found abundantly in the genomes and are normally uniformly distributed, as well as very variable and codominant. Each locus is defined by a pair of primers; therefore, the information can be easily interchanged between laboratories. The SSR markers were also found to be useful for cultivar identification and phenetic relationship assessment, revealing advantages due to higher reproducibility over other commonly employed PCR-based methods, namely RAPD and AFLP (Goulão and Oliveira 2001).

Microsatellites are the variations or mutations of very short DNA sequences. Differences between individuals consist in variations in the number of

repetitions of the same sequence. The origin of such polymorphism can be due to sliding in the DNA replication (Zane et al. 2002). Other possible causes of polymorphism generation consist in different types of mutations as deletions and insertions that will also change the size of the microsatellite. The biggest problem is that it needs hi-resolution gels to obtain all the information contained, plus the great initial effort that is required to clone and sequence the primers. The use of microsatellites for genotyping can occasionally be complicated by the preferential amplification of some alleles if the optimal temperature is not used (Fernández-Fernández et al. 2004). In addition, if there are mutations in the matching zones of the primers, the result could be null alleles. This circumstance has already been pointed out by Callen et al. (1993) as a possible problem associated with the use of microsatellite markers. If undetected, a null allele would merely result in that individual being scored as a homozygot, therefore resulting in a loss of information (Marinoni et al. 2003).

A European project formed by 11 European groups, named HiDRAS (*hi*gh-quality *di*sease *r*esistant *a*pples for a *s*ustainable agriculture), is aimed at the identification of the genetic factors that control fruit quality. They are looking for molecular markers linked to fruit quality and pathogen resistance to improve 'marker-assisted selection' (Gianfranceschi and Soglio 2004).

5.3.1 Isoenzymes

The first studies in isoenzymes for clonal identification were done by Chyi and Weeden (1984), Menendez et al. (1986a, b), and Weeden and Lamb (1985), contributing later to the first genetic maps (Lawson et al. 1995). Gardiner et al. (1996) used isoenzymes, RAPD's, and RFLP's to find out the parents of cv. Braeburn, very common in New Zeeland.

Heritability of seven isoenzymes was studied by Chevreau et al. (1985); later on, Weeden and Lamb (1987) published the genetics and linkage between 19 isoenzymes loci and Manganaris (1989) 13 isoenzymes. Got was suggested to be linked to the incompatibility gene *S* (Manganaris and Alston 1987) and proposed its use as a marker. Manganaris and Alston (1988a) found a linkage between acid phosphatase with the gene *ENP-1* (endopeptidase) and the lethal gene in apple *GENE 1*. Genetics of Lap isoenzyme and its variations between main apple cultivars were shown by Manganaris and Alston (1992a). The highly polymorphic peroxidase was used for cultivar identification (Manganaris and Alston 1992b). Locus Pgm-1 was closely linked to the *Vf* scab-resistance gene (Manganaris et al. 1994). Heretability and linkage of Got with other isoenzymes was published by Manganaris and Alston (1988b) and its use for cultivar and rootstock identifications in 1989. Lawson et al. (1995) found that blooming was correlated with Prx-c. Linkage to woolly aphid resistance was studied using stylar ribonucleases and Got-1 (Tobutt et al. 2000).

The following isoenzymes have been defined: Aconitase (Aco-1, Aco-2, Aco-3, Aco-4) by Hemmat et al. (1994) and Chevreau et al. (1999); acid phosphatase

(Acp-1, Acp-2 Ap, Acp-3, Acp-4, Acp-5) by Manganaris and Alston (1988b), Chevreau and Laurens (1987), and Hemmat et al. (1994); alcohol dehydrogenase (Adh-2) by Manganaris (1989); Catechol oxidase (Ctx-1 Co-1, Ctx-2 Co-2) by Chevreau et al. (1999); diaphorase (Dia-1, Dia-2, Dia-5, Dia-6) by Chevreau et al. (1999) and Weeden and Lamb (1987); endopeptidase (Enp-1 Enp) by Chevreau and Laurens (1987); esterase, esterase cathodic (Est-1, Est-2, Est-3, Est-4, Est-c) by Manganaris and Alston (1992a), Chevreau et al. (1985), Pereira-Lorenzo et al. (2003); formate dehydrogenase (Fdh-1 Fdh, Fdh-2) by Hemmat et al. (1994) and Chevreau et al. (1999); glutamate oxaloacetate (Got-1, Got-2, Got-4, Got-5 Aat-5) by Manganaris and Alston (1987, 1988a) and Chevreau et al. (1999); glucosephosphate isomerase (cytosolic and plastid) (Gpi-cl, Gpi-p) by Weeden and Lamb (1987); isocitrate dehydrogenase (Idh-1, Idh-2, Idh-3) by Chevreau (1984), Weeden and Lamb (1987) and Manganaris (1989); leucine aminopeptidase (Lap-1, Lap-2, Lap-3, Lap-4) by Manganaris and Alston (1992b); malate dehydrogenase (Mdh-1, Mdh-2, Mdh-3, Mdh-4) by Manganaris (1989) and Weeden and Lamb (1987); malic enzyme (Me-1) by Weeden and Lamb (1987); 6-phosphogluconate dehydrogenase (Pgd-1 Pgd-c1, Pgd-2 Pgd-c2, Pgd-3 PGD-3) by Weeden and Lamb (1987) and Manganaris (1989); phosphoglucose isomerase (Pgi-3 PGI-3) by Manganaris (1989) and Pereira-Lorenzo et al. (2003); phosphoglucomutase (Pgm1 Pgm-p1, Pgm-2, Pgm-3, Pgm-4, Pgm-5) by Weeden and Lamb (1987), Manganaris (1989) and Pereira-Lorenzo et al. (2003); peroxidase (Prx-1, Prx-2, Prx-3, Prx-4, Prx-7, Prx-C1, Prx-C2) by Manganaris and Alston (1992c); shikimate dehydrogenase (Skd Skdh) by Hemmat et al. (1994); superoxide dismutase (Sod-1, Sod-2, Sod-3, Sod-4, Sod-5) by Manganaris and Alston (1987) and Chevreau et al. (1999); and triosephosphate isomerase (Tpi-1 Tpi-pl, Tpi-3, Tpi-3, Tpi-5 Tpi-c2) by Weeden and Lamb (1987).

Batlle and Alston (1994) pointed out the interest of using isozymes for tracing the transference of the resistance to mildew (*P. leucotricha* (Ell. et Ev.) Salm.) between *M. hupehensis* and cultivated apples. James and Evans (2004) used a set of microsatellites, AFLP and RAPD primers, to identify markers linked to mildew resistance. In recent years, genetic markers have been developed for a number of resistance genes, such as for apple scab (*V. inaequalis*). Bus et al. (2004) who work with microsatellites presented the discovery of a new scab-resistance gene (*Vh8*) that maps to linkage group 2. RAPD markers, located in a chromosomal region that confers scab resistance to apples, were used to screen *Malus* germplasm accessions. The following results were discussed in relation to the introgression of resistance loci together with marker-assisted selection (King et al. 1999).

Durel et al. (2004) studied five mapping populations looking for partial scab resistance against several races of *V. inaequalis*. They worked with SSR and AFLP to test each population, and the genetic maps for both parents of each population were constructed. The occurrence of new virulent races that are able to overcome the *Vf* resistance (Benaouf and Parisi 2000) has initiated the search for new resistance sources, as well as further genetic and molecular

characterization of the already known strong resistances. So, Boudichevskaia et al. in 2004 developed molecular markers for *Vr1*, a scab-resistance factor. A selection attending to adverse factors of the apple tree using molecular markers was emphasized by Tartarini et al. (1997). Screening of seedlings for peroxidase allozyme variation was found to be a reliable method to preselect apple dwarf types (Tang and Zhang 1992). The availability of molecular markers and genetic linkage maps enables the detection and the analysis of major resistance genes as well as of QTL contributing to the resistance of a genotype (Liebhard et al. 2003).

Allozyme analysis indicated that the genetic integrity of native populations of *Malus* was effectively protected against gene flow from cultivated apple (Dickson et al. 1991). Recommendations for the efficient sampling of genetic diversity from natural populations of *M. sieversii* were formulated based on an analysis of population structure using allozyme markers (Lamboy et al. 1996). Isoenzymes were also used to study hybridization and species differentiation by Dickson et al. (1991). In 2002, the morphologic and isoenzymatic characterization of the collection of native apple tree cultivars gathered in the 'Centro de Investigaciones Agrarias de Mabegondo' (CIAM) was published (Pereira-Lorenzo et al. 2002). In this work, 408 accessions of apple tree were studied and compared with 32 nonnative commercial varieties. The same study allowed obtaining results with respect to the genetic variability in the collection of the CIAM (Pereira-Lorenzo et al. 2003). The variability level found has been elevated, since 86% of the introductions are original, which are maintained in the Germplasm Bank. The rest of the accessions turned out to be repetitions of others and even of nonnative commercial and extensively cultivated varieties, such as 'Reina de Reinetas' and 'Reineta Blanca'.

Also a recent study has been published about the isoenzymatic variability of the germplasm of native apple tree cultivars that has been established in the last years by the Public University of Navarre using seven isoenzymatic systems (Itoiz and Royo 2003).

Isoenzymes were employed to determine the genetic structure of 202 trees representing *M. sylvestris* from different regions in western Germany, and the results were compared to similar data on 321 old and new cultivars of *M x domestica* (Wagner et al. 2004). The results of this study indicate that gene flow in either direction has been minimal.

5.3.2 Microsatellites

The first study with microsatellites in apple tree was published by Guilford et al. (1997), who described the first three SSRs in apple tree. Gianfranceschi et al. (1998) extended it to 17. Later, Liebhard et al. (2002) elevated the number of SSR to 140 and used them to propose a map of global linkage in apple tree.

Costa et al. (2004) developed an SSR marker associated with fruit firmness. Kenis and Keulemans (2004) worked with RFLP and microsatellites in order to study the genetic control of tree architecture in apple.

Hokanson et al. (1998) screened 66 *Malus x domestica* Borkh accessions from the USDA-ARS Plant Genetic Resources Unit core collection with a set of eight SSR. Later, they made the characterization of another 142 accessions, which represents an extensive range of *Malus* species and derived hybrids (Hokanson et al. 2001).

The genetic variation within and between wild apple samples and cultivated apple trees was investigated with AFLP and SSR to develop a genetics conservation program for the endangered wild apple (*M. sylvestris*) in Belgium (Coart et al. 2003). One hundred and forty-two French local cultivars were screened with nine SSR markers to get a characterization of the apple genetic resources in France (Lawrens et al. 2004).

SSRs were used in genetic identification by Guilford et al. (1997), Hokanson et al. (1998, 2001), Liebhard et al. (2002), Kitahara et al. (2005), Pereira-Lorenzo et al. (2007), Cabe et al. (2005), and Oraguzie et al. (2005). SSRs were used in the study of the genetic variation in wild apple by Coart et al. (2003). They were also used for the study of haploids by Hofer et al. (2002) and the tea crabapple *M. hupehensis* by Benson et al. (2001).

SSRs allowed the development of genetic maps by Liebhard et al. (2002), Hemmat et al. (2003), and Gianfranceschi et al. (1998). They were also used to study *Vf* scab-resistance region by Vinatzer et al. (2004). Vinatzer et al. (2004) localized two microsatellites markers for the *Vf* gene, and they were also used to verify the genealogical tree of the *Vf* cultivar 'Florina'. Hemmat et al. (2002) provided SSR markers for *Vr* and *Vx*, mapping those genes in R12740-7A accession, and Patocchi et al. (2004) for *Vr2*.

5.3.3 Other Markers

Segregation patterns of AFLP markers have been studied by Li et al. (2004). AFLPs have been used for the construction of genetic maps including the *Vf* gene for scab resistance (Xu and Korban 2000, 2002) and for cultivar identification (Tignon et al. 2000; Tignon et al. 2001a; Tignon et al. 2001b).

AFLPs have been combined with other PCR-based molecular markers and FISH for mapping resistance to aphids (*Sd1*) by Cevik and King (2002a,b). In addition, AFLPs, RAPDs, SSRs, and SCAR were used to set up a saturated reference map by Liebhard et al. (2002).

Other combined markers used to define genetic maps have been isoenzymes and RAPDs (Hemmat et al. 1994), RFLPs, RAPDs, isozymes, SSRs, and SCARs (Maliepaard et al. 1998) with the location of the scab-resistance gene (*Vf*), resistance to rosy apple aphid (*Sd1*), self-incompatibility (*S*), and fruit acidity (*Ma*). Combination of SSRs and ISSRs were used for identification by Goulão and Oliveira (2001).

Unspecific markers as RAPDs have been profusely used to localize molecular markers for fruit skin color (Cheng et al. 1996), molecular markers for powdery mildew resistance (Markussen et al. 1995; Dunemann et al. 1999), *Vf*, *Vm* genes (Cheng et al. 1998; Hemmat et al. 1998), to verify apomictic seedlings (Ur-Rahman et al. 1997), and to build linkage maps (Conner et al. 1997).

SCARs have been defined for scab genes *Vm* and *Vf* (Cheng et al. 1998; Shupert et al. 2004), powdery mildew gene *Pl1* (*P. leucotricha* (Ell. & Ev.) E.S. Salmon) (Evans and James 2003), and columnar *Co* gene (Kim et al. 2003).

Combinations of AFLPs, RAPDs, and SSRs provided scab markers for *Vr2* gene (Patocchi et al. 2004); RAPDs and SSRs were used to study columnar *Co* gene (Hemmat et al. 1997); RAPDs and SSRs defined markers for scab *Vr* and *Vx* genes (Hemmat et al. 2002); RAPDs and SCARs were used to study *Vf* gene (Tartarini et al. 1999); and RAPDs, SCARs, and SSRs for scab-resistance *Vbj* gene (Gygax 2004). Different markers, such as RAPDs, isoenzymes in combination with morphology, were used in cultivar identification by Royo and Itoiz (2004) and SSRs and ISSRs by Goulão and Oliveira (2001).

Gene tagging with DNA markers has been used to follow the inheritance of individual genes, such as those conferring scab resistance, *Vm* (Cheng et al. 1998).

5.3.4 Cultivar Classification by Biotechnological Methods

Cultivar classification was one of the main aspects in breeding since it allowed differentiating cultivars for different purposes.

Before 20th century, agronomists tried to classify varieties by painting them in very fine detail, as can be seen in many 17th and 18th century canvasses. During the last century, most apple classification studies focused on giving detailed descriptions, which were based mainly on fruit morphology and agronomic characteristics. Descriptions were made by pomologists who carefully detailed the main characteristics that defined each cultivar, adding along frequently a precise handmade picture illustration (Guinea 1957).

It was not until the second half of the 20th century that apple cultivars began to be classified attending systematic guidelines as those set by IBPGR (1982) or UPOV (1974). IPGRI is focused in the conservation of genetic resources and includes more details on the origin of the cultivars (passport data), as well as relevant cytological information and isoenzymes. UPOV (1974) is mainly focused on cultivar protection and, therefore, can be used as a guideline to distinguish cultivars with the purpose of obtaining new patents. Both guidelines provide main and secondary characteristics with different variation levels according to the total variability found previously between apple cultivars. An update including more molecular markers is needed, such as microsatellites that are very accurate in distinguishing cultivars, although it is understood that morphology is required to define them.

Systematics allowed applying statistics to the evaluation in order to get strong evidence on such variations between cultivars. Statistics applied to classifications are mainly based on means, variance, ANOVA, principal component analysis, and cluster analyses (Pereira-Lorenzo et al. 2003; Royo and Itoiz 2004). Within these guidelines, we can understand how difficult differentiation is when a high number of accessions are involved, and molecular markers are excellent instruments in differentiating these (Oraguzie et al. 2005). New classifications complemented with the use of molecular markers enable to identify genetic variations avoiding environmental influence. An extensive study was developed in a Spanish collection of local cultivars (408 accessions) in order to know the main origins of variability, find out duplications, and classify them. Cultivar description is fundamental for the management of germplasm banks, and in the Spanish collection it allowed to remove 53 duplications (Pereira-Lorenzo et al. 2002, 2003). Spanish cultivars were studied during 3 years for phenology, fruit, leave, and flower based on UPOV (1974) and IBPGR (1982) descriptors. A total of 89 characteristics were evaluated and split into 279 variables. The code used in the descriptor lists is indicated in brackets. Three steps were defined for morphology: (1) variability description; (2) variance analysis; and (3) multivariate analysis. To increase the capacity of discrimination, high-discriminant isoenzyme systems were developed.

Rootstocks have been classified by different authors and we can find excellent descriptions by Masseron (1989) and Webster and Wertheim (2003). Some very detailed descriptions of cultivars from different countries have been made, like from France (Boré and Fleckinger 1997), Spain (Guinea 1957; Coque et al. 1996; Pereira-Lorenzo et al. 2002, 2003), the UK (Morgan and Richards 1993), and the USA (Beach et al. 1905).

Different molecular markers have been used for rootstock and cultivar identification: (1) isoenzymes (Manganaris 1989; Weeden and Lamb 1985; Pereira-Lorenzo et al. 2003); (2) AFLPs (Tignon et al. 2000, 2001a); (3) SSRs (Oraguzie et al. 2005; Pereira-Lorenzo et al. 2007); (4) ISSRs (Goulão and Oliveira 2001); and (5) RAPDs (Royo and Itoiz 2004).

QTLs have been studied for branching habit, vegetative bud break, reproductive bud break, bloom time, and root suckering using molecular markers (Lawson et al. 1995), in combination with RAPDs to study juvenile tree growth (Conner et al. 1998). QTLs for stem diameter, plant height increment, leaf size, bloom traits, juvenile phase, and fruit characteristics have been evaluated by Liebhard et al. (2003), fruit quality by King et al. (2000), scab resistance by Calenge et al. (2004), and powdery mildew resistance by Stankiewicz-Kosyl et al. (2005).

5.4 Resistance to Pests and Diseases

Complete reviews have been made by Grove et al. (2003) and Beers et al. (2003).

Resistance to fire blight has been one of the main objectives in the Geneva breeding program (Norelli et al. 2003a; Norelli et al. 2003b). Susceptibility of

'M9' and 'M26' rootstocks has encouraged the selection of resistant rootstocks, such as 'G.16', 'G.30', or 'G.65' (Grove et al. 2003).

Scab (*V. inaequalis*) is one of the main high-cost diseases for growers. As the result of breeding programs developed during the last 50 years, several cultivars including resistance from *M. floribunda* 821 have been released. However, growers do not incorporate them to the new orchards due to their inferior quality. It is not clear if resistance is due to a cluster of genes or to a major *Vf* gene (Barbieri et al. 2003). A total of eight genes for scab resistance have been described—*Va, Vb, Vbj, Vf, Vfn, Vm, Vr,* and *Vr2* (Dayton and Williams 1968; Barbieri et al. 2003; Patocchi et al. 1999a, b, 2004; Xu and Korban 2002). *Vm* is a resistant gene to scab derived from *Malus x atrosanguinea* 804 and *Malus micromalus* 245-38 (Cheng et al. 1998). A selection identified in the USA from an open pollination seed obtained in Russia denominated as R12740-7A was identified by Hemmat et al. (2002) as carrying *Vr* and *Vx* genes. GMAL 2473 is an apple scab-resistant selection thought to carry the resistance gene *Vr2* (Patocchi et al. 2004).

New cultivars including durable resistance are needed for organic growing and integrated fruit production (IFP). Some cultivars with known resistance to scab are being evaluated now (Sandskar and Gustafsson 2004). Some of them are (resistance gene in brackets) as follows: (1) from Canada, 'MacFree' (*Vf*), 'Novaspy'® (*Vf*), 'Nova Easygro' (*Vr*), and 'Richelieu' (*Vf*); (2) from the Czech Republic, 'Selena'® (*Vf*), 'Topaz'® and 'Vanda' (*Vf*); (3) from France, 'Baujade' (*Vf*), 'Florina'® or 'Priam' (*Vf*), 'Judaine' (*Vf*), and 'Judeline' (*Vf*); (4) from Germany, 'Reglindis'® (*Va*), 'Reka'® (*Vr*), 'Regia'® (*Vr*), 'Rewena'®, 'Rebella'®, 'Retina'®, 'Resi'®, 'Releika'®, 'Renora'®, 'Remo'® (*Vf*), 'Ahrista'® (*Vf*), 'Gerlinde'® (*Vf*), and others; (5) from the USA, 'Liberty'® (*Vf*), 'Prima'® (*Vf*), 'Freedom'® (*Vf* + *Vr*), and 'Priscilla' (*Vf*); (6) from Holland 'Santana'® (*Vf*); (7) from Switzerland 'Ariwa'® (*Vf*); and (8) from Russia, 'Antonovka kamienna' (*Va*), 'Imrus' (*Vf*), and 'Antonovka Pamtorotuka' (Va).

Powdery mildew (*P. leucotricha* (Ell. & Ev.) E.S. Salmon) reduces tree photosynthesis and transpiration and may produce partial defoliation (Grove et al. 2003). Between cultivars and rootstocks, we can find different susceptibility, with 'Golden Delicious' being less susceptible than 'Gala' or 'Granny Smith'. 'Malling-Merton' rootstocks are very susceptible (Janick et al. 1996). *Pl-1, Pl-2, Pl-w*, and *Pl-d* genes for resistance have been described by Knight and Alston (1968), Dunemann et al. (1999), Markussen et al. (1995), Alston et al. (2000), Batlle and Alston (1996), Batlle (1993), Dayton (1977), Korban and Dayton (1983), and the precursors *Pr-Pl-1* by Batlle (1993) and Batlle and Alston (1996).

WAA resistance of rootstocks has been reviewed by Sandanayaka et al. (2003). Three major WAA resistant genes have been identified—*Er1* (Knight et al. 1962; King et al. 1991), *Er2* (King et al. 1991), and *Er3* (Sandanayaka et al. 2003), which are carried by the apple cultivars 'Northern Spy', 'Robusta 5', and 'Aotea', respectively. *Er1* and *Er2* each had a higher level of resistance and these resistance factors appeared to be in the phloem tissue (Sandanayaka et al. 2003).

As we explained about rootstocks, WAAs encouraged an important breeding program in 1952 that produced two frequently used rootstocks 'M.106' and 'M.111', resistant to *E. lanigerum* (Masseron, 1989). They were selected in 1952 from crosses between 'Northen Spy' and some selections of the serial EM.

The rosy leaf-curling aphid (*Dysaphis devecta* Wlk.) causes severe leaf curl with conspicuous red galls. Alston and Briggs (1968, 1970 and 1977) and Roche et al. (1997) described three aphid biotypes and four resistance genes providing resistance to these biotypes: (1) *Sd-1* gene for biotypes 1 and 2 from 'Cox's Orange Pippin'; (2) *Sd-2* gene for biotype 1 derived from 'Northern Spy'; and (3) *Sd-3* gene for biotype 3 derived from *M. robusta* and *M. zumi*. Cevik and King (2002a,b) showed that Sdh-1 and Sdh-2 loci are tightly linked. *Pr-Sd* has been described as precursor of *Sd* genes by Alston and Briggs (1977).

Breeding programs to combine different resistances with good fruit quality and with high and regular yield are very important. An excellent model for such a complex breeding program is the German apple breeding work at Dresden-Pillnitz. The first aim of this program was to breed both good fruit quality and high yield. All clones with high susceptibility to scab and mildew were eliminated in field evaluations. The program developed the 'Pi-series' of apple cultivars ('Pi' = Pillnitz), which included 'Pinova'®, 'Pilot'®, 'Piros'®, and others. 'Pinova'® and its red mutation 'Evelina' are two of the most interesting cultivars of the future.

In scab-resistance breeding, the cultivar 'Antonovka kamienna' was used at first as a polygenic scab-resistant source (Schmidt 1938), and later the *M. floribunda* and other wild species with different resistance sources (*Vf, Vm, VA*) were also involved. The resistance breeding program was extended in Pillnitz for mildew, fire blight, bacterial canker, red spider mite, and abiotic damage, such as winter frost and spring frosts. The results are the cultivars of the 'Re-series' ('Re' = Resistance) including 'Remo'®, 'Rebella'®, 'Rewena'®, 'Regia'®, 'Reglindis'®, and others. These cultivars have good fruit quality, early and high cropping, and show resistance to scab and to some extent to other fungal and bacterial diseases (Fischer and Fischer 1996; Fischer 1994, 2000).

One of the most important results of the Pillnitz apple resistance breeding program was the selection of a number of cultivars with resistance to economically important diseases using conventional recombinant breeding methods. The advanced Pillnitz resistant cultivars have been tested under a wide range of environmental conditions. They demonstrated their ability to maintain their resistance and provide fruit suitable either for fresh market and/or processing. With their resistance properties, they are suitable for organic fruit production and IFP. Triple and multiple resistant cultivars are selected with resistance to scab, mildew, and fire blight within the Re-cultivars™ 'Remo'®, 'Rewena'®, 'Regia'®, and 'Rebella'®. 'Rebella'® was found to have resistance not only to fungi and fire blight but also to bacterial canker, red spider mite, apple aphids, and abiotic damages. All other cultivars have a different level of multiple resistances (Table 7). These multiple resistances can be transmitted to the offspring by classical recombination breeding and requires no genetic engineering.

Table 7 Multiple resistances in the Pillnitz Re-cultivars™

Re-cultivar™	Scab	Source of resistance	Mildew	Fire blight	Bacterial canker	Red spider mite	Spring frost	Winter freeze
Reanda	R[1]	Vf	LR	R	LS	S	R	LS
Rebella	LR	Vf	R	LR	R	R	R	R
Regine	LR	Vf	LR	R	LR	R	R	R
Releika	LR	Vf	LS	LR	R	R	R	S
Relinda	LR	Vf	LR	LS	R	S	LR	R
Remo	LR	Vf	R	LR	LS	LS	R	R
Rene	LR	Vf	S	R	LR	S	R	LS
Renora	R	Vf	LR	LS	LS	LS	LR	LR
Resi	LR	Vf	LS	LR	R	S	R	S
Retina	LR	Vf	LR	LS	LS	LR	R	S
Rewena	R	Vf	R	R	R	LS	R	LS
Realka	R	Vr	S	R	LS	LS	S	LS
Regia	R	Vr	R	R	LR	LS	LS	R
Reka	R	Vr	LR	LS	R	S	S	LR
Releta	R	Vr	S	LS	R	LS	LS	LS
Remura	R	Vr	LR	LS	LS	S	LS	R
Reglindis	LR	V_A	LR	LR	LS	R	R	R

[1] R: resistant; LR: low resistance; LS: low susceptibility; S: susceptible

5.4.1 Overcoming of the *Vf* Scab Resistance

The *Vf* scab resistance is overcome under different wet conditions especially in northern Europe. Scab develops and results in consecutive infections during the summer, if no fungicides have been applied. Scab on *Vf* resistant cultivars has been observed at Ahrensburg, North Germany, since 1984 (Krüger 1999). At different degrees of intensity, some *Vf* Re-cultivars™ carried weak infections, sometimes with defensive reactions. At another location in northern Germany, the infection occurred as a primary infection at an early stage before blossom opening and caused severe symptoms on peduncles, calyx, and, somewhat later, on petals. Foliar infections spread from 'Gerlinde'® (*Vf*) to the neighboring 'Ecolette'® (*Vf*), 'Topaz'® (*Vf*), and 'Rebella'® (*Vf*). At another location near the Baltic Sea, scab was observed in 2000 only on 'Prima' and 'Ecolette'® and in 2001 on all tested resistant cultivars (Höhne 2001; Fischer et al. 2005). In spite of the lability of the *Vf* scab resistance, these multiple-resistant cultivars are now of interest because of their stable fire blight resistance (Fischer 1994, Fischer and Richter 1999). One year with scab infection is not synonymous with regular yearly infection.

At other locations in the middle and south of Germany, resistant cultivars remained free of infection till now. The resistant cultivars produced defensive reactions, with the exception of 'Reglindis'® (V^A), considered field resistant, with very light sporulation lesions. This reaction is typical for polygenic V^A resistance. The ones remaining free of scab under all conditions were 'Reka' (*Vr*), 'Recolor'® (V^A + *Vf*), and 'Regia'® (*Vr*).

Apparently, the entire genetic background of the resistant cultivars is the cause of differences in resistance stability. Probably, not only one *Vf* gene exists but also three closely related genes. If one or two genes are absent, the resistance is instable (Benaouf and Parisi 2000; Lespinasse 2001). The results indicate that a number of resistant cultivars remained healthy in their respective locations, which allows a rather stable resistance to be assumed. This group includes 'Reglindis'® (V^A), 'Reka' (*Vr*), 'Regia'® (*Vr*), 'Renora'® (*Vf*), 'Relinda'® (*Vf*), 'Reanda'® (*Vf*), and 'Rewena'® (*Vf*). However, the future needs new cultivars with two or three different resistance sources in order to stabilize healthiness in the fields, if the *Vf* gene is overcome and does not work any longer.

What we can do? For durability of scab resistance in the field, we recommend (1) no 'monoculture' with *Vf* cultivars; (2) tolerance of a slight leaf infection on polygenic/oligogenic resistant cultivars to preserve the stability of the host–pathogen system (using V^A- or *Vr* cultivars like 'Reglindis'® or 'Reka' in change with *Vf* cultivars); and (3) three fungicide sprays in early spring would be enough to control infections. In the following seasons, some cultural measures were employed successfully, such as using urea sprays during and after leaf drop in autumn in order to promote leaf rotting or collecting mechanically infected leafs by means of large vacuum cleaners (Triloff 2006).

After these treatments, no primary scab infections were observed in the following spring. Results so far show that a very significant reduction of fungicide spray applications, up to 80%, can be achieved without significant scab and mildew infections in orchards (Fischer and Fischer 2002; Fischer et al. 2005).

In apple breeding, there is still an aim to bring together improvements in fruit quality + yield + resistance to different pathogens in new cultivars. Another new challenge is to establish a lasting resistance in field cultivation, based on observations carried on in different parts of Europe on the breakdown in monogenic scab-resistance sources from *M. floribunda* (Weibel et al. 1997; Fischer et al. 1998; Fischer and Dunemann, 2000; Fischer and Fischer 2002). The stabilization of the *Vf* resistance in the field by breeding needs (1) promoting the breeding of cultivars with two or more resistance sources by pyramiding different resistance genes; and (2) using more cultivars with polygenic scab resistance in combination breeding programs (Lespinasse 2001).

6 Conclusions

Earnest efforts have been made by different researchers in order to understand the apple tree: (1) the botanical aspects; (2) breeding and selection of cultivars and rootstocks; (3) variability and genetic resources; (4) knowledge of pests and diseases; (5) breeding for resistance; (6) technical and genetic aspects in the improvement of the crop; and (7) development of powerful techniques such as molecular markers to assist breeding programs.

However, some questions remain and they should be addressed in the future: (1) the convenience of a more variety of cultivars that combine high-quality, good postharvest conservation and resistance to main pests and diseases; (2) dwarf or semidwarf rootstocks with good resistance to cold winters, excellent compatibility, and enough vigor to avoid trellis (columnar habit in new cultivars should help to it); (3) a phylogeny review, including new tools such as molecular markers, should be afforded in order to understand better this complex genus; and (4) a complete world evaluation of apple genetic resources that would allow to maintain a high variability avoiding an elevated number of repetitions.

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Apricot Breeding

Hannél Ham

1 Introduction

Apricots are most popular temperate fruit trees (Faust et al. 1998; Layne et al. 1996), bearing delicious and multipurpose fruits. The fruit can be dried, canned, juiced, preserved (by salting or smoke), made into jam, and also used medicinally. Additionally, the seed of some cultivars are edible, tasting like almonds, while the tree can be used as an ornamental plant (Faust et al. 1998). Most apricot cultivars belong to the species *Prunus armeniaca*, which is endemic to China (Layne et al. 1996).

Mediterranean countries account for 95% of the total fresh apricot market, and the fruits are mainly imported and consumed by the European community (Mahanoglu et al. 1995; Faust et al. 1998; Ham and Smith 2006). Although apricots are geographically widespread, they have not become economically viable except in areas with very specific climatic conditions. Apricots grow best in mountainous regions with a hot, dry summer and uniform, cold winter (Layne et al. 1996; Ham and Smith 2001). Although apricot is a temperate zone fruit, some cultivars and types can be grown in subtropical areas. In such areas, by using the low-chilling apricot cultivars, the fruits can be harvested early in the season (Kaska et al. 1995; Gulcan 1997).

2 Botany

The apricot belongs to the family Rosaceae and the genus *Prunus*. It is an interfertile diploid species with eight pairs of chromosomes ($2n = 16$). Most cultivated apricots belong to the species *P. armeniaca* L. Closely related species are *P. brigantiaca* (Briancon apricot from the French Alps); *P. ansu; P. mume* (Japanese apricot); *P. sibinica; P. mandshurica*, and *P. dasycarpa* (black

H. Ham
ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599 South Africa
e-mail: hamh@arc.agric.za

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apricot). *P. dasycarpa* is a natural hybrid between *P. armeniaca* and *P. cerasifera* (Bailey and Hough 1975; Layne et al. 1996; Faust et al. 1998; Hurtado et al. 2006). The Rosaceae family ranks as the third most agronomical important plant family in temperate regions and includes fruits such as apple, peach, nectarine, plums, cherries, and strawberries (Dirlewanger et al. 2002; Sosinski et al. 2000; Hurtado et al. 2006).

Apricots are indigenous to north-western China. The trees grow in solid stands as forests on dry mountain slopes. It has originated in the north-eastern areas of China, through the centuries crossed the plains of Asia and arrived in Europe before the birth of Christ. It was only after many centuries that its cultivation extended into the Americas (Monastra and De Salvador 1995; Gulcan 1997). Favorable areas for apricots in China are found between 35° and 40° N altitude, at altitudes of 700–1500 m where rainfall is less than 500 mm per year, with minimum temperatures as low as −33°C. The main growing areas are China, the Mediterranean European Countries, Turkey, and USA. Most of these countries have apricot breeding programs, but despite this, very little information concerning the genetics of apricot is available (Hurtado et al. 2006).

Apricots have a wide range of phenotypes and genotypes that can be recognized especially with regard to ecological adaptation. Its diffusion is, however, limited to certain pedoclimatic areas of the world and this has given rise to an intense process of selection and natural adaptation, which has resulted in the creation of many different strains of genotypes and phenotypes (Monastra and De Salvador 1995; Gulcan 1997). Eight different ecogeographical groups have been identified. The cultivars of Europe, North America, South Africa, and Australia belong to the 'European' group. The European group is the youngest and least variable of the four most important groups (Burgos et al. 1997).

3 World Production

The apricot is considered by many to be one of the most delectable of tree fruits (Layne et al. 1996), and one of the few temperate fruit crops not affected by production surplus (Bassi 1997). The apricot is one of a small number of species, which, because of its versatile uses (fresh market and processing) has the possibility of expanding even further and without suffering from the recurrent market crises of many others species. With approximately 2.6 million tons in 2004 (Table 1), apricot production is far below that of apples or peaches. Widely distributed, apricot production is centered in the Mediterranean area that possesses 95% of the total fresh apricot market, and these fruits are imported and consumed mainly by the European Countries (Monastra and De Salvador 1995; Faust et al. 1998; Audergon 1995; Mahanoglu et al. 1995; Yildiz et al. 1997; OABS 2005).

Turkey is the leading producing country both in fresh and dried apricots. In the 2004 season, 10 countries in very different geographical areas accounted for

Table 1 World production of apricots for 2004 season (OABS 2005)

Ranking	Country	Production (Metric tons)
1	Turkey	440,000
2	Iran, Islamic Republic of	280,000
3	Italy	209,000
4	France	157,400
5	Pakistan	135,000
6	Spain	125,700
7	Syrain Arab Republic	100,000
7	Ukraine	100,000
9	Morocco	97,950
10	USA	86,680
11	China	83,000
12	Russian Federation	80,000
13	Egypt	72,000
14	Algeria	70,000
14	Greece	70,000
16	South Africa	68,000
17	Romania	42,000
17	Uzbekistan	42,000
19	Lebanon	30,000
19	Tajikistan	30,000
21	Tunisia	26,000
22	Argentina	25,000
23	Chile	22,000
24	Serbia and Montenegro	20,000
25	Australia	19,742
26	Libyan Arab Jamahiriya	17,000
27	Czech Republic	16,800
28	Azerbaijan, Republic of	15,000
28	Hungary	15,000
29	Kyrgystan	14,500
30	Rest of world	115,714
TOTAL		2,625,486

66% of the world production: Turkey (16.8%), Iran (10.7%), Italy (8%), France (6%), Pakistan (5%), Spain (4.8%), Syrian Arab Republic (3.8%), Ukraine (3.8%), Morocco (3.7%), and USA (3.4%) (OABS 2005).

4 Nutritional Value

Agriculture provides not only employment but also food for an increasing population in rural as well as urban areas. Fruits (especially apricots) are not staple food, but have high nutritional value when eaten fresh (Gulcan 1997).

Apricots are considered by many to be one of the most delicious and popular of multipurpose temperate tree fruits. The fruit can be dried, canned, juiced, preserved (by salting or smoke), made into jam and used medicinally (Faust et al. 1998; Layne et al. 1996). The dietary value of a 100-g edible apricot portion (two medium size fruit) is summarized by Mark's Fruit Crops (2003) as mentioned below:

		% of US RDA	
Water (%)	85	Vitamin A	54
Calories	51	Thiamin, B1	2.1
Protein (%)	1.0	Riboflavin, B2	2.5
Fat (%)	0.2	Niacin	3.3
Carbohydrates (%)	13	Vitamin C	22
Crude fiber (%)	0.6	Calcium	2.1
		Phosphorus	2.9
		Iron	5.0
		Potassium	6.0

5 Breeding Objectives

Breeding the 'perfect' apricot will result in a satisfied breeder, producer, exporter, and consumer. In order to obtain this, there are universal apricot breeding objectives, based on tree characteristics (rootstocks, tree vigor, growth habit, and productivity), floral biology (flowering date, intensity, and fertility), fruit characteristics (maturity, size, firmness, color, taste), disease resistance, and climatic adaptation (cold hardiness, chilling requirement, and spring frost) (Audergon 1995; Egea et al. 1995; Gulcan et al. 1995; Dosba 2003; Bassi 2006; Ham and Smith 2006).

Apricot culture mostly depends on the interaction between the climate, soil conditions, and the scion cultivar. Production, fruit quality, and time of harvest will be affected by these three factors (Ayanoglu and Kaska 1995). The effect of soil conditions can be minimized first by selecting the best suitable rootstock for the soil conditions (salinity, nematodes, etc.) and then by correct orchard management practices. Breeding for climatic adaptation, better fruit quality, and disease resistance is much more complicated.

5.1 Climatic Adaptation

Apricots need plenty of sunshine, rain or irrigation water, and fertile soils. They are better adapted to areas where summers are hot and dry with little atmospheric humidity (Gulcan et al. 1995; Kaska 1997). Furthermore, apricots need a cold period during winter to stimulate budbreak and release dormancy (Küden and Son 1997). Insufficient winter chilling can cause abnormalities

such as delayed foliation resulting in poor fruit set (Bartolini et al. 1997; Faust et al. 1998), whereas few or no fruits will develop after an extremely warm winter (Yildiz et al. 1997; Bassi 2006). Delayed foliation, however, is not always an indication of lack of adaptability. If temperatures are too high for an extended period of time during the dormant period, differentiation stops and bud drop may occur. On the other hand, warm temperatures favor the development of pollen grains. When temperature remains low, there is little, if any, pollen development (Layne et al. 1996; Alburquerque et al. 2006). Rain prior to harvest can cause cracking of fruit due to absorption of water through the skin of the fruit. This can damage some or even most of the fruit and allow the entry of fungi and subsequent rotting (Gulcan et al. 1995). High UV exposure and high light intensities linked to high temperatures, or a combination of these factors can cause sunburn as well as pit burn (softening and discoloring around the pit) due to the lack of oxygen. These problems can be addressed by using vigorous rootstocks and pruning manipulations ensuring added shading to the fruit (Huysamer 1997). In the warm apricot growing regions, this is a serious problem where there are several days of high temperatures (over 40°C) just prior to harvest (Bailey and Hough 1975; Layne et al. 1996).

5.2 Fruit Quality

In general, fruit quality refers to size, time of harvest, sugar, taste, aroma, color (flesh and skin), red blush, firmness, and production (Egea and Burgos 1999). Many breeders aim to extend the period of supply for the fresh apricot market by creating new cultivars, which ripen earlier or later than the existing commercial varieties, but most of the selections have poor eating quality or are unattractive (Tzoneva and Tsonev 2000; Bassi 2006).

Production is influenced by a variety of factors, such as self-compatibility, alternate bearing, frost resistance orchard management (pruning, thinning, irrigation, fertilization, planting densities, etc.), pest and disease activities, and climate adaptation of scion cultivar and rootstocks (compatibility with scion, adapted to climate and soil conditions) (Bassi 2006).

5.3 Disease Resistance

Disease resistance can influence the fruit quality and production of a new cultivar and is primarily influenced by climatic conditions. Disease resistance can be achieved by breeding-resistant cultivars, natural control, or chemical control. The most common disease can be summarized here (Bailey and Hough 1975; Layne et al. 1996; Karayiannis 1995; Audergon et al. 1995; Dosba 2003; Bassi 2006; Myrta et al. 2006).

5.3.1 Bacterial Cancer (*Pseudomonas syringae*)

Bacterial cancer causes severe damage (cankers on the woody organs of the tree) in apricot orchards planted in stony, sandy, and acid soils (pH 5–6) and exposed to cold winters and humid climates. The lesions produced by exposure to cold temperatures could be easily infected and develop cankers that may lead to loss of branches, scaffolds, or even the whole tree.

5.3.2 Bacterial Leaf Spot (*Xanthomonas pruni*)

Bacterial leaf spot causes severe defoliation and fruit spotting in some seasons, weakens the tree, and renders many fruits unmarketable. Older leaves and fruit bearing trees are more susceptible, and spreading is more severe in warm and humid climates.

5.3.3 Brown Rot (*Monilinia laxa, M. fructigena, and M. fructicola*)

Brown rot, although not so dangerous from a strictly epidemiological point of view, can cause notable economic damages and is strongly influenced by the climatic conditions (wet) in two critical phonological phases of the tree: bloom and ripening. An inadequate spraying program can result in destruction of flowers and most of the young shoots.

5.3.4 Apoplexy (Dieback of Shoots and Branches)

Apoplexy is caused by bacteriosis due to environmental and/or pathological factors. There is no effective cure since it enters the tree and moves around in the sap stream. The best solution is to cut off a shoot or branch the moment it wilts. The infected wood will be stained brown and the unaffected wood will be pale and wholesome.

5.3.5 Blossom Blight

Blossom blight can affect production (tons per hectare) and fruit quality due to abnormal pollination conditions. It can be controlled by chemical spraying.

5.3.6 Chlorotic Leaf Roll

Chlorotic leaf roll is caused by a phytoplasm similar to the agent of ‘flavescence doree’ in vineyards and causes a progressive decline of the tree due to the obstruction of the sap vessels. It is transmitted by grafting and insects.

5.3.7 Sharka or Plum Pox Virus

Plum pox virus (PPV) is currently the most destructive disease in Europe and is spreading worldwide. Different groups of PPV strains have been identified, for example, Marcus (M), Dideron (D), Cherry (C), and El Amar (EA). The M strain seems to spread faster and more readily in the field. Trees affected by this virus show a decrease in productivity. The long latent time after infection, the uneven distribution of the virus in the tree, and the speed of multiplication by means of the many species of aphids make this probably the most dangerous disease.

5.3.8 Other Apricot Viruses

Ilarviruses (Prunus nectoric ringspot virus (PNRSV), Prune dwarf virus (PDV), apple mosaic virus (ApMV)), genus *Trichovirus* (apple chlorotic leaf spot virus (ACLSV)), and Viroids (hop stunt viroid (HSVd)) can cause economical losses.

6 Breeding Systems

Breeding can be divided into two main groups (biotechnology and conventional). Biotechnology breeding mainly consists of molecular assisted selection (MAS) and/or genetic transformation. It is a very popular breeding system for breeding PPV resistance (Bassi 2006). Although MAS can save time for the conventional breeder, it will never replace conventional breeding. Conventional breeding includes the following breeding systems.

6.1 Varietal and Selection Crossing

The crossing of two or three phenotypes is equally promising and evaluation of the resulting progeny for desirable traits (Bailey and Hough 1975; Bassi 2006).

6.2 Modified Backcrossing

Certain characters, such as disease resistance, cold hardiness, and late blooming, can be effectively incorporated with other desirable pomological characters by selecting the best parental material and evaluating the progeny for the desirable traits (Bailey and Hough 1975; Layne et al. 1996).

6.3 Interspecific Hybridization

Crosses between plums and apricots (Plumcot) and some characteristics in other *Prunus* species, such as later blooming, greater disease resistance, cold

hardiness, and modified tree types, would be of value for regional adaptation if they could be incorporated into apricots (Layne et al. 1996; Bassi 2006).

6.4 Mutation Breeding

The manipulation of irradiated material, by selecting the best parental material, allows for early recognition of the desired mutants in the progeny under evaluation (Bailey and Hough 1975).

6.5 Hand Pollination

Conventional hand pollination is done by collecting flowers in the field within 2–3 hours after sunrise. The flowers may be in the balloon stage or beginning to open so long as the anthers have not begun to dehisce. The pollen from a desirable parent cultivar (male tree) can then be used to make crosses on another parent cultivar (female tree). The progeny is then evaluated for the desirable traits (Bailey and Hough 1975; Layne et al. 1996).

7 Molecular Markers

Horticultural biotechnology offers an exciting approach in meeting two particular challenges faced by the food and export industry: sustainable horticulture and improved quality of fresh produce and processed products (Andrea 1992). Biotechnology has obvious implications as a new tool that can be employed in apricot breeding. Potential areas for its application include regeneration and micropropagation, virus elimination, and genetic improvement, which could include somaclonal variation, protoplast culture and fusion, embryo rescue, and haploid induction. Recombinant DNA technology might also be employed to carry out genetic transformation and gene characterization (Layne et al. 1996).

Apricot breeding is time-consuming, especially for fruit-specific characters as the trees must grow for at least 3–4 years before it bears fruit (Dirlewanger et al. 1998). Early selection with markers would be particularly interesting in apricots given the long generation period. Molecular markers are currently being applied in genetic diversity studies because, unlike morphological characteristics, they are not affected by environmental variation. For example, amplified fragment length polymorphism (AFLP) has been used to successfully detect genetic variation among and within populations to determine the genetic structure and differentiation of populations (Ricciardi et al. 2002).

Molecular markers can be used as tools in the different steps of the breeding and propagation processes, including marker-assisted selection of characters of simple and complex inheritance, cultivar identification, pedigree analysis, or identification of distant germplasm sources (Aranzana et al. 2002). Molecular markers linked to these traits are of great value for the identification and selection of plant genotypes with the desired characters long before the traits are expressed. Molecular markers linkage maps are useful for localizing important genes controlling both qualitative and quantitative traits in numerous plant species (Dirlewanger et al. 1998).

In most cases, the genetic engineering process involves inserting a gene from one organism into the genetic code of another organism. Since all DNA has the same basic structure, the only barriers to this selective transfer are our ability to identify desirable genes and the availability of appropriate transfer systems. Genetic manipulation can be used to transfer genes between almost any number of organisms, while in conventional breeding, the transfer of genes is only possible between sexually compatible organisms, usually within the same species (Andrea 1992).

Fruit crops are vegetatively reproduced so that the genotype of a single individual of a cultivar should be identical to the rest. This simplifies the process of cultivar identification with markers. The high degree of polymorphism of molecular markers such as microsatellite markers (Single Sequence Repeats (SSR)) and AFLPs provide efficient tools for identification and revealing genetic diversity among apricot germplasm (Arus 2006; Struss et al. 2006).

Conventional breeding is also a lengthy process, especially for organisms with long generation times, but it could not be replaced by genetic engineering. With conventional plant breeding, there are several constraints on the types and degree of changes possible. Genetic engineering may be able to speed up some breeding programs, and it can potentially overcome some of the constraints in conventional breeding, such as sexual incompatibility, but will not get rid of the requirement for a favorable, equilibrated genetic background for the expression of any new gene (Andrea 1992).

Conventional plant breeders cross genotypes carrying different horticultural traits with the aim of producing recombinant individuals that combine good traits of both the parents. Since this trait is related to fruit characteristics, the breeder must wait several years until the newly developed seedlings have passed their juvenile phase. Only after this period, seedlings start to bear fruit and it becomes possible to determine if the desirable trait is present in the progeny. However, if the gene controlling the phenotypic trait was tagged with a marker, which shows no or very low recombination with the gene, it would have enabled the breeder to make an early selection among seedlings, discarding those that do not carry the marker and propagate only the ones who did carry the relevant gene. This is why MAS is becoming increasingly popular in crop plant breeding (Testolin 2003).

In the 1980s, when scientists worked with isozymes, and MAS was still a new concept, restriction fragment length polymorphism (RFLP) was used. RFLPs

were based on the use of DNA probes, plotted against genomic DNA digested with restriction enzymes and electrophoresis on agarose gels. RFLPs have been extensively used in stone fruit genetics. As the MAS technique developed, scientists started using random amplified polymorphic DNAs (RAPDs) and AFLPs as new markers (Testolin 2003; Arus 2006; Struss et al. 2006). AFLP is a DNA fingerprinting technique that detects DNA restriction fragments by means of polymerase chain reaction (PCR) amplification. It is a very reliable and robust technique, which is unaffected by small variations in amplification parameters. The high marker densities that can be obtained with AFLP are an essential characteristic of the technology: a typical AFLP fingerprint contains between 50 and 100 amplified fragments, of which up to 80% may serve as genetic markers. Moreover, AFLP technology requires no sequence information or probe collections prior to the generation of AFLP fingerprints. AFLP markers usually exhibit Mendelian inheritance, indicating that they are unique DNA fragments. It is a quick, reproducible, robust, relatively inexpensive but reliable procedure to distinguish between, for example, a group of tetraploid potato cultivars (Lambert 1998; Carter and Brock 1980; Hagen et al. 2002; Wang et al. 2002; Testolin 2003; Arus 2006; Struss et al. 2006).

Although AFLP is a powerful, cost-effective method for identifying DNA polymorphism, AFLP markers are generally dominant, requiring conversion to sequence tagged sites (STSs) for application in comparative mapping studies and for practical use in marker-assisted selection. On the other hand, SSR markers are PCR-based and exhibit codominant inheritance (Aranzana et al. 2002; Wang et al. 2002; Sosinski et al. 2000).

Microsatellite markers are suitable for comparative genetic studies, and can facilitate the integration of genetic maps both within the Rosaceae and across wider taxonomic boundaries. SSRs have emerged as an important system of molecular markers (Sosinski et al. 2000) and the best choice in mapping peach (*Prunus persica*) genome. However, developing an SSR map is very time-consuming and expensive, and most SSRs are not specifically linked to gene loci of immediate interest (Wang et al. 2002; Testolin 2003; Arus 2006; Struss et al. 2006).

8 Inheritance of Characteristics

Apricot has a great deal of genetic variability (Bailey and Hough 1975), but information on apricot traits' heritability is very scarce. However, for some important fruit traits, simple Mendelian heritability has already been described, viz., flesh color, pit adhesion, and skin fuzz (hairiness). There is still uncertainty about some characteristics, such as fruit size, color (skin and background), flavor, red blush, seed (sweet and bitter), and time of ripening that can be influenced by climatic conditions or polygenic control. The average performance of the progeny could be predicted on the basis of the phenotype of the parents (Bailey and Hough 1975; Layne et al. 1996; Bassi 2006).

9 Harvest and Postharvest

Apricots are perishable fruits, which are highly appreciated when the eating quality is superior. Apricots ripen and mature rapidly at ambient temperatures, and require careful and rapid handling after harvest to avoid serious losses. The expectations of consumers and fruit buyers (retailers, exporters) are very high, but they are often disappointed due to the external and internal quality of the apricots. Thus, the harvest maturity of apricots has a major influence on the postharvest storage quality of apricots and revenue earned from markets (Desphande and Salunkhe 1964; Ginsburg and Combrink 1972). Furthermore, different varieties have different features and handling protocols, and therefore, varying quality is often observed in the market caused by the lack of distributors' knowledge about shelf life and storage ability of the individual varieties (Lichou 1999).

The maturity at which apricots are picked, the precooling down temperature and period, as well as the storage temperature and period have an effect on the color, taste, and texture of the apricot. There are various methods in determining the right period for harvesting (e.g., free stone, background skin color, firmness, soluble content, acidity). An apricot picked too early can never reach a good quality because woolliness or gel breakdown, taste, sugar levels, and skin color can occur (Van Rhyn and Redelinghuys 1988; Jooste and Taylor 1999). In order to prevent this, it must be picked as close to optimum maturity as possible, to offer good-quality fruit to consumers (Lichou 1999). The storage life of apricots is often limited by excess water loss, which in other words means weight loss and softening, resulting in the total collapse of the mesocarp (Agar and Polat 1995).

Controlled-atmosphere (CA) storage is used when apricots are exported by ship from the southern to the northern hemisphere. CA reduces the respiration rate of fruit and thus extends the life of the product. In the case of pome fruit (apples), it is possible to double the storage life, but this does not necessarily hold true for stone fruit (Truter et al. 1994; McLaren et al. 1997) especially apricots. Sugar contents and acidity levels change until harvest, but only acid continues to evolve after picking. Cold storage usually slows down the ripening process of apricots by reducing ethylene (a gaseous hydrocarbon) levels. The concern with longer transport times (up to 5 weeks) is whether the fruit will store and retain its flavor successfully (McLaren et al. 1997).

Extension of the storage period is normally associated with an increase in the incidence of physiological disorders (e.g., decay, gel breakdown, internal breakdown, etc.) in fruit. Gel breakdown (a gelatinous breakdown of the mesocarp tissue surrounding the stone) is one of the main physiological disorders that occur in apricots (Truter et al. 1994; Jooste and Taylor 1999; De Klerk and Von Mollendorff 1994; Taylor and De Kock 1999). It is usually associated with overripeness and internal breakdown with long storage periods at low temperatures. In severe cases, the breakdown spreads toward the skin, changing from

translucent to a brown discoloration. This disorder can initiate in the orchard and is aggravated during cold storage, making the fruit unsuitable for sea export (Jooste and Taylor 1999; De Klerk and Von Mollendorff 1994).

Over-maturity develops with similar symptoms, but with the difference that it spreads from the exocarp to the endocarp. The appearance of gel breakdown varies between seasons, while ripe apricots are more subject to gel breakdown than unripe apricots at harvest (De Klerk and Von Mollendorff 1994; Taylor and De Kock 1999).

10 Orchard Management

The traditional planting density for apricots was less than 500 trees per hectare (trees/ha) with an open vase training system. The modern approach for planting of apricots is toward higher planting densities (600–1300 trees/ha). However, very high densities (more than 1300 trees/ha) result in low-quality fruit and difficulty in orchard management (pruning and thinning). As the number of trees/ha increases, the yield also increases but at the expense of quality (size, color, etc.). Thus, all the necessary cultural operations become progressively more difficult by planting more trees per hectare (Monastra and De Salvador 1995; Southwick and Weis 1998). Therefore, training systems such as Palmetto, Spindle, and Tatura, which control the tree size more effectively, were introduced (Vachun 1995).

The apricot has a high rate of growth during its first years, which is influenced by the cultivar, rootstock, and climatic conditions. Without proper pruning practices, the production and fruit quality will be influenced. Apart from the type of pruning, the time in which it is carried out is of prime importance in relation to the possible phytosanitary problems of the species. Pruning carried out during the winter period can cause fungus attacks, with serious damage to the branches or even to the entire plant. To avoid such problems and the appearance of gummosis, it is recommended to prune in the summer after harvest, resulting in better fruit quality. Pruning should always be light, but sufficient enough to allow adequate sunlight penetration into the tree canopy for proper maturation of shoots (Monastra and De Salvador 1995; Xiloyannis et al. 2006).

The younger and more vigorous the tree (with many long, 1-year shoots full of feathers), the lighter should be the pruning. If on the other hand the plant is weak with short shoots full of flower buds and/or full of spurs and short twigs, it should be pruned vigorously in order to renew 25–30% of the wood. If the production is based on the spurs from which the best fruit is derived, it is necessary to cut back in such a manner as to renew 25% of the fruiting branches every 2–3 years (Monastra and De Salvador 1995). The sterilization of pruning equipment is of the utmost importance to prevent the spread of diseases.

An effective and balanced fertilization program is absolutely necessary for high-yield and good-quality fruits. Specific knowledge of the nutritional needs of the apricot is fragmentary and incomplete. However, noteworthy amounts of the macroelements nitrogen and potassium, together with the microelements iron and zinc, are annually removed by the apricot tree during the growth season (Monastra and De Salvador 1995). Nitrogen is the element that results in the most rapid and evident effects on tree growth, while phosphorous and potassium can counterbalance negative effects, favoring the differentiation of the flowers and increasing the yield (Monastra and De Salvador 1995; Xiloyannis et al. 2006).

Fruit thinning is considered a normal technique in orchard management of apricots to guarantee fruit of high quality, uniformity in ripening, and consistency in production. The best results are obtained with hand thinning carried out 20–30 days after full bloom, with increments of 36% in fruit weight, while thinning operations carried out after stone hardening are not satisfactory (Monastra and De Salvador 1995).

11 Rootstocks

Rootstocks are almost as important, if not more, as scion cultivars. The interaction between the rootstock and the scion cultivar has a direct impact on the yield, fruit quality, and eventually on the profitability of the scion cultivars as well as premature mortalities of trees. It is, therefore, of utmost importance to select the best suitable rootstock when the establishment of a new apricot orchard is planned. Further, to perform optimally, a combination of correct orchard practices and rootstocks adaptable to the unique soil and climate conditions of an orchard is a key aspect in being competitive and successful in deciduous fruit farming. A rootstock cannot be changed during the lifetime of an apricot orchard because the function is long-term and complex (Vachun 1995; Ercisli and Guleryliz 1995).

Apricot rootstocks are limited to light, well–drained, and neutral-pH soils that are relatively low in lime. In areas with heavy soils, a higher water table, the occurrence of long rainy periods, root asphyxiation, and oxygen stress limit the use of some apricot rootstocks. Other characteristics of soil–plant interaction that dictate the rootstock selection are the buffering ability for soil pH and nutrient uptake efficiency (Southwick and Weis 1998).

Rootstocks are propagated from seedlings (seed collected from certified apricot cultivars), clonal (rooted cuttings from certified apricot rootstocks), and the use of intermediate stocks (apricot rootstock grafted on plum or peach rootstock) for certain soil conditions where apricot rootstocks do not perform well. The seedling and clone rootstocks, unlike the intermediate stock, will be affected directly by below ground issues such as soil-borne diseases, insects, water logging, low fertility, etc. However, intermediate stocks (Fig. 1) have shown to provide

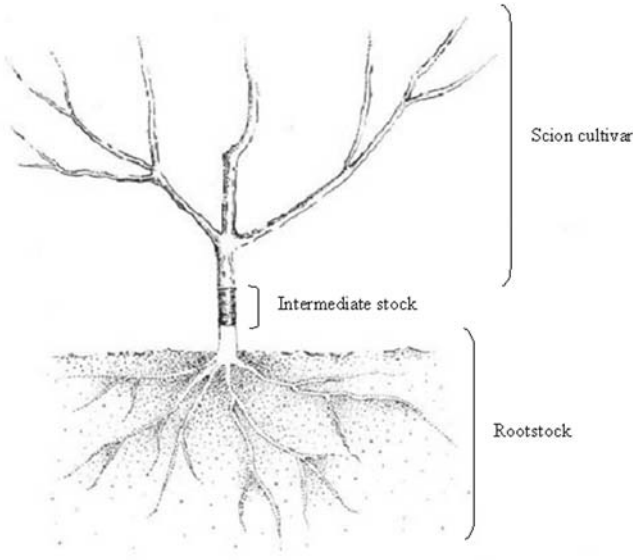

Fig. 1 A diagrammatic representation of an intermediate stock

hardier trunks, control vigor, delay bloom and fruit maturation, and improve fruit quality if the interaction between the clonal and intermediate stock cultivars is optimum. But intermediate stocks are more time-consuming and costly, while issues such as graft incompatibility and virus sensitivity remain the same for intermediate stocks as for rootstocks (Beckman 2003; Dosba 2003).

Breeding of new apricot rootstocks is a very time-consuming exercise. In order to ensure that well-adapted and evaluated apricot rootstocks are released for cultivation, they need to betolerant or resistant to nematodes, diseases, insects, edaphic factors, with good propagation (rooting ability), and considering hortological and pomological aspects (Layne et al. 1996; Southwick and Weis 1998; Beckman 2003; Dosba 2003; Vachun 1995; Monastra and De Salvador 1995). The evaluation for the interaction between the root system and the canopy of the scion cultivar must be optimized to increase tree efficiency. The selection of interspecific hybrids for rootstocks is one solution to meet complex objectives in a rootstock-breeding program (Dosba 2003).

11.1 Nematodes

One of the most intensely active areas of stone fruit rootstock breeding is the breeding for nematode resistance. Most production areas around the world have significant problems with one or more species of nematodes. Nematodes

are microscopic, wormlike organisms that attack the roots of plants resulting in a decrease of nutrient and water uptake due to damaged roots (Dosba 2003; Beckman 2003). More commonly found nematodes according to Dosba (2003) include the followings.

11.1.1 Root-Knot (*Meloidogyne* spp.)

Endoparasites feed on the inside of the roots that make visible knots on the outside of the roots as an infection symptom. While not all production areas are infested with the same species, several are commonly found worldwide, including *M. incognita, M. javanica,* and *M. arenaria*. Other less common species causing significant problems in certain localities are *M. hapla* and *M. hispanica*.

11.1.2 Ring (*Mesocriconema xenoplax*)

An ectoparasite (feeding on the outside of the roots) makes it much more difficult to prove resistance.

11.1.3 Lesion (*Pratylenchus* spp.)

An endoparasite feeds on the inside of the roots. Two species dominate most research interests, *Pratylenchus vulnus* and *P. penetrans*.

11.1.4 Dagger (*Xiphinema* spp.)

An ectoparasite feeds on the outside of the roots. The principal species are *Xiphinema americanum* and *X. rivesi*.

11.2 Disease and Insect Resistance

Diseases and insect infestations can be controlled by either chemical spraying or breeding of resistant stone fruit rootstocks. However, it is of the utmost importance that when pruning the scion cultivars, the pruning equipment is sterilized to minimize the spreading of diseases in an orchard. Furthermore, when the first signs of diseases of insect infestations are noted, the infected material needs to be removed from the orchard and burnt. There are several soil-borne diseases that can attack stone fruit (including apricots) rootstocks, which can affect the fruit of the scion rootstock and furthermore result in economical losses. It is summarized by Beckman (2003).

11.2.1 Fungi

There are three *Armillaria* species (*A. mellea, A. tabescens*, *and A. ostoyae*) that attack stone fruit rootstocks, especially apricots and peaches. Plum species seems to have a tolerant or resistant reaction toward *Armillaria* species and are therefore used as intermediate stocks in conjunction with an apricot rootstock and scion cultivar. *Phytophthora* is often the cause of tree decline and death on waterlogged sites as a secondary infection. However, when the trees are subjected to both *Phytophthora* and water logging, the damage is much worse. Several species of *Phytophthora* have shown to be pathogenic in *Prunus*. Other soil-borne disease such as *Fusarium, Phymatotrichum, Rhizoctonia, Rosellinia*, and *Verticillium* can also attack the apricot rootstock.

11.2.2 Bacterial Cancer

Bacterial cancer is incited by *P. syringae* and can be a significant problem in all stone fruits. It can be controlled by chemical spraying, removal, and burning of infested trees, or by breeding resistant stone fruit rootstocks.

11.2.3 Viruses

Viruses such as tomato ringspot (TmRSV), PNRSV, and PDV can cause economical losses (production and fruit quality) due to the necessary removal and burning of infected material. These viruses are spread by nematode, and therefore when nematode control is applied, it can help to control these viruses but only to a limit, depending on the severity and damaged to trees.

11.2.4 Peach Tree Borers

This is the most important insect pest attacking fruit tree rootstocks. It can be controlled by normal good orchard management practices.

11.3 Edaphic Factors

Edaphic factors refer to the soil factors such as the chemical, physical, and biological properties of soil that influence the life of organisms or plants. The main edaphic factors include water content, organic content, texture and pH, or factors that influence the efficient uptake of nutrients and water by the root system. The most common factors, according to Beckman (2003), are described next.

11.3.1 Calcareous or Limy Soils

Soil with a high pH (>8) will influence the uptake of minerals (such as iron) by the plant.

11.3.2 Salt Tolerance

Soil with a high salinity has a high pH (>8) and, therefore, will influence the uptake of nutrients and water by the plant. This is generally a localized problem but may increase in importance as agricultural water resources shrink due to demands placed on them by human populations.

11.3.3 Water Logging Tolerance

Phytophthora infections are normally associated with water logging conditions, making it difficult to determine if the plant died of water logging stress or *Phytophthora* infections.

11.3.4 Drought Tolerance

Water stress is a problem not only in areas with limited rainfall (irrigation water shortages), but also in areas of significant annual rainfall resulting in highly variable periods of unusual drought due to global climatic changes. It also has an effect on the water table level and thus influencing the regularity of irrigation in orchards, which has a negative effect on certain physiological processes of the apricot tree.

11.3.5 Nutrition and Low Fertility

Numerous studies have demonstrated that rootstocks have an influence on foliar nutrient content of stone fruit scion varieties. For example, rootstocks that are sensitive to calcareous conditions may be also sensitive to lime-induced iron chlorosis. This might affect the photosynthesis ability of the scion and result in low fruit production.

11.3.6 Cold Hardiness

Low temperature stress involves the hardiness of the rootstock itself. In extreme northern latitudes with adequate snow pack, this is normally not a problem. However, in locations where winter snowfall is inadequate or comes after the occurrence of extreme low temperatures, rootstock damage can be a threat to the survival of the tree. Breeding for this character can be complicated by the interaction of secondary factors, such as various bark and wood diseases that enter cold-damaged areas. This phenomenon does not occur in most Mediterranean climate countries such as South Africa, Southern Europe, etc.

11.4 Horticultural Influence

The success of an orchard is characterized by the influence of the soil, climate, and rootstock on the scion. Therefore, newly bred rootstock cultivars with tolerance or resistance to different traits (such as ring nematodes) will not succeed without promoting superior hortological performance to the scion. High, reliable, uniform production of premium-quality fruit is essential for economic survival. Rootstocks can have significant influence on the pomological attributes of the scion cultivar. Challenging economic conditions, including increased material, labor, and land costs, market competition, and overproduction, have furthermore increased the importance of production efficiency issues (Beckman 2003).

11.4.1 Vigor

Several new stone fruit production systems have been introduced in recent years, including Palmetto, Fusetta, Perpendicular-V, Spindle, Solaxe, Spanish bush, and others. On high-fertility sites with vigorous scion cultivars, some reduction in vigor is highly desirable if only for reduced pruning, thinning, and picking costs. As an added benefit, vigor reductions are often accompanied by improved fruit quality, in particular for the development of red blush, and increased size and higher sugars due to reduced shading (Beckman 2003).

11.4.2 Bloom Time

The potential for a rootstock to either promote or delay blooming probably deserves more attention than it receives. Such bloom date alterations can translate into proportional harvest date alterations, and/or can be important for spring frost susceptibility or avoidance (Beckman 2003).

11.4.3 Spring Shock Syndrome

This is a recently reported phenomenon, the cause of which is still not understood completely. For example, during atypically cool springs in low-chill areas of Australia when soils are slow to warm, peaches on high-chill rootstocks lag well behind those on low-chill rootstocks (Beckman 2003).

11.4.4 Graft Compatibility

Incompatible responses include low yield, breaking off at the graft union, and high prevalence of bacterial canker. The development of dwarfing rootstocks or spur cultivars has led to problems of graft incompatibility (Dosba 2003).

12 Constraints for Breeding

Bassi (1997, 2006) address some of the problems that need to be solved through breeding programs in the near future. They are

- the diversity and heterozygosity of the apricot germplasm,
- adaptation to the environment, which is hard to manage through standard crossing and selection due to time constraints and the speed of climate change,
- flower bud differentiation,
- production (fresh and processed),
- fruit quality (size, firmness, aroma, flavor, skin color, sugar, and shape),
- disease resistance (e.g., PPV),
- MAS in combination with conventional breeding,
- mapping of the apricot genome,
- resistant or tolerant rootstocks and grafting compatibility with scion cultivars, and satisfying consumer preferences on an ongoing basis.

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Citrus Breeding

Fred G. Gmitter Jr., Jaya R. Soneji, and Madhugiri Nageswara Rao

1 Introduction

With a total world production of 105.4 million tons, citrus is one of the world's most important fruit crops (FAO 2006). Its importance to agriculture and the world's economy is demonstrated by its wide distribution and large-scale production (Soost and Roose 1996). It is grown throughout the tropical and subtropical regions of the world where the winter temperatures are sufficiently moderate for tree survival and enough water is available for its growth (Gmitter et al. 1992). The best fruit quality is achieved under subtropical conditions. The most significant citrus-producing regions are in the Americas (Brazil, USA, Argentina, and Mexico primarily), the Mediterranean basin (Southern Europe, Southwest Asia, and North Africa), Asia (including China, India, and Japan) and South Africa. Citrus industries in many production areas generate substantial regional revenue. Brazil, USA, China, Mexico, and Spain are the five largest citrus producers in the world (Table 1, FAO 2006). Sweet orange is grown on about 3.6 million ha in 114 countries with an approximate production of 64 million tons (t) with Brazil being the largest producer. The world production of grapefruits and pummelo is 4 million t and is grown in 74 countries on about 264,000 ha. USA is the largest producer of grapefruits and pummelo. China produces 38% of 18 million t of mandarins and their hybrids produced in the world. Lemons and limes are produced in 94 countries on about 0.8 million ha with a production of approximately 7.7 million t with Mexico being the largest producer. Brazil and Florida (USA) produce citrus fruit primarily destined for the juice or concentrate market, while China, Mexico, Spain, and California (USA) produce primarily fresh-market fruit. Citrus is valued as a fresh fruit and is also processed into juice, or added to dishes and beverages.

F.G. Gmitter Jr.
University of Florida, Institute of Food and Agricultural Sciences, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850, USA
e-mail: fgg@crec.ifas.ufl.edu

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Table 1 Top ten *Citrus*-producing countries in the world

Country	Production (000 MT)
Brazil	20,576
China	10,395
USA	14,985
Mexico	6490
Spain	5103
Italy	3285
Argentina	2430
Egypt	2688
Turkey	2450
South Africa	1683

(Source: FAO 2006)

Citrus is widely produced in dooryard plantings for personal and local consumption as well.

2 Origin and Domestication

The center of origin and diversity of citrus and its related genera is generally considered to be Southeast Asia, especially East India, North Burma, and Southwest China, possibly ranging from Northeastern India eastward through the Malay Archipelago, north into China and Japan, and south to Australia (Tanaka 1954; Webber 1967; Scora 1975, 1988; Gmitter and Hu 1990; Soost and Roose 1996). The oldest known reference to citrus appears in Sanskrit literature that dates to before 800 BC followed by descriptions in Chinese, Greek, and Roman literature (Webber 1967; Scora 1975). The first citrus fruit to arrive in Europe was the citron, followed by the sour orange, lemon, and sweet orange (Webber 1967). The colonial expansion of Europe introduced citrus to the rest of the world, including the Americas, South Africa, and Australia. Recent evidence supports the view that Yunnan Province in the Southwest China may be the center of origin as a diversity of species is found there (Gmitter and Hu 1990). Although the exact routes of dispersion of citrus from its origin are unknown, the network of rivers in the Yunnan Province area could have provided a natural route for dispersal to the south (Sauer 1993). Most scion and rootstock cultivars that are widely grown in the main commercial producing areas of the world originated as either chance seedling selections or bud sport mutations (Hodgson 1967). In recent decades, however, with increasing capabilities for genetic improvement of citrus afforded by new technologies, there have been increasing numbers of new cultivars released from breeding and genetic improvement approaches, and that trend will accelerate in the coming decade.

3 Botanical Aspects

3.1 Taxonomy

The genus *Citrus* belongs to the subtribe Citrinae, tribe Citreaea, subfamily Aurantioideae of the family Rutaceae. This genus may be further divided into two subgenera (*Citrus* and *Papeda*), based on leaf, flower, and fruit properties. The evolution of modern citrus cultivars and their diversity has been addressed (Swingle and Reece 1967). On the basis of morphological characteristics, studies have been carried out on the relationships between genera and species. This has led to the formulation of numerous classification systems. The most commonly used citrus classifications are by Swingle (Swingle and Reece 1967) and Tanaka (Tanaka 1977). In the genus *Citrus*, Swingle recognized only 16 species, whereas Tanaka recognized 162 species. The difference in these two systems reflected opposing theories on what degree of morphological difference justified species status and whether presumed hybrids among naturally occurring forms should be given species status (Soost and Roose 1996). A comprehensive phylogenetic study by Barrett and Rhodes (1976) evaluated 146 morphological and biochemical tree, leaf, flower, and fruit characteristics; they concluded that there were three biological species of the so-called edible citrus types, with several other wild species. However, Scora (1975) suggested that only three citrus types, citron (*C. medica*), mandarin (*C. reticulata*), and pummelo (*C. grandis*; now *C. maxima*), constituted valid species and viewed all others as introgressions of these ancestral forms. Recently, molecular marker studies have supported this hypothesis (Nicolosi et al. 2000). Lime (*C. aurantifolia*), *C. micrantha*, and *C. halmii* are also included in the list of 'true' citrus species by many researchers. Papeda is a group of *Citrus* species (*C. ichangensis, C. micrantha, C. latipes, C. celebica, C. hystrix, and C. macroptera*) having inedible fruit with acrid oil droplets in the juice vesicles. Understanding taxonomy, phylogenetic relationships, and genetic variability in citrus is critical for determining genetic relationships, characterizing germplasm, controlling genetic erosion, designing sampling strategies or core collections, establishing breeding programs, and registering new cultivars (Herrero et al. 1996). The phylogeny and genetic origin of important species of citrus has been investigated using molecular markers (Nicolosi et al. 2000; Moore 2001; Berkeley et al. 2006); however, these studies have not been able to clearly differentiate all the species. Hence, there is a need for additional taxonomic studies to further clarify the taxonomic distinctions.

3.2 Geographical Distribution

Citrus is commercially grown in the tropical and subtropical regions around the world, primarily between the latitudes of 40°N to 40°S, from equatorial,

hot-humid climates through warm-subtropical and even cooler maritime climates (Spiegel-Roy and Goldschmidt 1996). The warm, humid semitropical climate enables the production of large quantities of fruit suitable for processing and is also suitable for grapefruit production. The cool, coastal valleys are suitable for the production of lemons. High-quality sweet oranges are grown in the intermediate valleys, which have semi-arid, subtropical climates. The desert valleys have hot, arid climates suitable for the production of grapefruit and certain types of lemons and mandarins. There is some overlap in the types of fruits produced in the different growing areas (CDCGC 2004).

3.3 Morphology

Citrus plants are small to medium sized, spreading, evergreen trees with thorny shoots, growing to about 2–15 m tall. Distinctive growth habits, ranging from spreading to upright to weeping, are observed among various species and cultivars. Most species are single-trunked with very hard wood (Manner et al. 2006). The main branches diverge from the trunk at 60–120 cm above the ground, depending on whether they are seedling trees or grafted to rootstocks, which is the most common commercial production approach (Schneider 1968). The general branching system of cultivated varieties of citrus gives the top or crown of the tree a more or less spherical shape. Trees produced from seeds tend to have more thorns than trees produced from grafting (Manner et al. 2006). The stem is green, with unifoliate, alternate leaves. Leaf shape varies from lance-shaped to round, and the size varies from 4 to 10 cm in length. Some types of leaves possess more or less broadly winged petioles. Leaves contain citrus oil glands, which are released when crushed (Manner et al. 2006). Flowers are fragrant, borne solitary or in short cymes in the axils of the leaves or in small lateral or terminal inflorescences. The flowers are usually white but sometimes pink or purple pigmented (in lemon and citron, and their hybrids with other citrus), perfect with five thick, linear, strap-shaped petals and four- to five-lobed sepals. The petals alternate with the sepals (Schneider 1968). There are usually four times as many stamens as petals. The stamens are polyadelphous, cohering toward the bases in a few bundles. The yellow, four-lobed anthers surround the pistil at or near the level of the stigma (Spiegel-Roy and Goldschmidt 1996). The ovary is superior and composed of 6–14 carpels joined to each other and to a central axis (Soost and Roose 1996). The ovary has a prominent but usually deciduous style containing as many tubes as there are cells in the ovary. The fruit is a hesperidium berry. It is a true fruit arising through growth and development of the ovary, consisting of a variable number of united, radially arranged carpels (Spiegel-Roy and Goldschmidt 1996). The forms and sizes of fruits vary from globose to oblong and oblate. They are highly fragrant and full of flavor and juice. The outer rind is known as flavedo (exocarp and endocarp).

It is covered with tiny pockets containing aromatic oils. The albedo (mesocarp) covers the endocarp. The pulp (endocarp) is divided into 10–14 sections containing specialized structures, the juice vesicles (sacs). They are separated by thin septa (Manner et al. 2006).

4 Reproductive Biology

4.1 Flowering

Although seasonal conditions may cause citrus to bloom at various times, the main blooming period of citrus trees in subtropical climates is in the spring (Erickson 1968). The environmental and endogenous control of flower bud differentiation is quite complex and varies considerably from one species to another (Spiegel-Roy and Goldschmidt 1996). The induction of flower buds begins with a cessation of vegetative growth during the winter rest period in subtropics or dry periods in tropical regions (Davies and Albrigo 1994). Flowering shoots are most commonly produced in citrus on woody twigs of the previous year's spring flush but may also be borne on younger, summer flush twigs or on older wood (Spiegel-Roy and Goldschmidt 1996). The vegetative meristem undergoes histological and morphological changes to differentiate into a floral meristem (Davenport 1990). Cassin et al. (1969) demonstrated that cold or water stress are the primary inductive factors, with cold being the primary factor in subtropical climates and water stress in tropical climates. To induce a significant number of flower buds, temperature below 20°C or drought periods longer than 45–50 days are required (Cassin et al. 1969). Induction of flowering by low temperatures or water stress was correlated with an increase in leaf ammonia content (Lovatt et al. 1988). The low-temperature induction of flowering in citrus has been shown to be accompanied by a decrease in endogenous gibberellic acids. Carbohydrate levels have been suggested as playing a role in the control of flowering. Girdling healthy trees in the early fall, and the consequent accumulation of carbohydrates above the girdle, usually increases flowering on healthy, nonjuvenile trees (Goldschmidt et al. 1985). Citrus trees usually bloom heavily; however, a comparatively small percentage of flowers produce mature fruit, since many flower buds and flowers drop before fruit set (Erickson 1968).

4.2 Pollination and Fertilization

Pollen is of sticky, adherent type. Honeybees are very effective for cross-pollination, but wind is a minor factor in its transfer from flower to flower. Development follows the usual course for angiosperm pollen. Production of

functional pollen varies tremendously among the various species as well as within the species. Several cultivars are pollen and ovule sterile. Most citrus cultivars are self-pollinated. The commercially important citrus species do not require cross-pollination generally (Davies and Albrigo 1994). Self-pollination can easily occur because of the proximity of anthers to stigma (Spiegel-Roy and Goldschmidt 1996). Some types are parthenocarpic, setting and maturing commercial crops of seedless fruit without fertilization and seed set. An exception to this is certain mandarin types and hybrids, which require cross-pollination (or in some cases self-pollination) to set fruit satisfactorily. Temperature also has a significant effect on pollination efficiency. The bee activity in the orchard is adversely affected when temperatures are below 12°C (Spiegel-Roy and Goldschmidt 1996). Even the pollen viability in some types, such as Satsuma mandarins, is dependent on the temperature (Soost and Roose 1996).

The germination and growth rates of the pollen grains which have landed on the stigma are enhanced at high temperatures (25–30°C). Low temperatures (<20°C) reduce or totally inhibited pollen germination. The arrangement and percentage of the planting of the pollenizer variety within the orchard are also important for successful pollination (Davies and Albrigo 1994). The pollen tube germinates and penetrates the embryo sac in the ovule. Fertilization occurs by fusion of a sperm (pollen) nucleus with an egg nucleus. Two microgametes are produced by the generative nucleus of the pollen. One microgamete fuses with the egg nucleus producing the zygote, while the other fuses with the two polar nuclei initiating the endosperm (Banerji 1954). Fertilization of the egg cell occurs 2 or 3 days after pollination under favorable conditions (Spiegel-Roy and Goldschmidt 1996).

4.3 Fruit Set

The term 'fruit set' is commonly used to describe the process through which the flower ovary adheres and becomes a fruit (Spiegel-Roy and Goldschmidt 1996). The appearance of a brown ring between the ovary and the style is the first sign of fruit set. The initial rate of fruit set, as observed soon after petal fall, is reduced markedly during the fruitlet abscission period. The percentage set expresses the ratio between the rather small, final number of fruit and the initial, very large number of flowers. Most of the commercially important cultivars produce around 100,000–200,000 flowers on a mature tree; however, the percentage of harvestable fruit is only 1–2% (Davies and Albrigo 1994). From flowering until 3–4 weeks postbloom, an initial drop period occurs. During this period, weak flowers and fruitlets with defective styles or ovaries, or flowers that did not receive sufficient pollination, abscise. The type of inflorescence and the position of individual flowers also affect fruit set. Most

of the fruit set on leafless inflorescence drop and the crop is eventually borne on leafy inflorescence (Goldschmidt and Monselise 1978). The leaves of the leafy inflorescence have been assumed to play a role in provision of photosynthate, mineral nutrients, or hormones to facilitate persistence of the young fruit (Spiegel-Roy and Goldschmidt 1996). The better water transport capacity of leafy inflorescence shoots may be responsible for the higher rate of fruit set (Erner 1989). Depending on the variety grown and the growing area, fruit development may take 5–18 months. When the fruits have reached maturity but prior to harvest, preharvest drop occurs. Spraying with a combination of gibberellic acid and 2,4-D can retard this fruit drop.

4.4 Fruit Ripening

Fruit growth of most citrus cultivars follows a sigmoid pattern, which can be divided into three phases (Bain 1958). Phase I is the cell division phase in which all the cells of the mature fruit are produced and the cells differentiate into various tissue types. It may be assumed to commence at fruit set. The increase in fruit size during this phase is mainly due to growth of the peel. Cell division appears to terminate in all fruit tissues, except the outermost flavedo layers and the tips of juice sacs, within 5–10 weeks after bloom (Spiegel-Roy and Goldschmidt 1996). The peel reaches its maximum width at or soon after the end of phase I. Phase II is the cell enlargement phase and produces a rapid increase in the fruit size. The percentage of total soluble solids also increases during this phase. The rapidly expanding pulp exerts pressure outward on the peel, which stretches and becomes increasingly thinner. During phase III or the maturation phase, the color of the peel begins to change from green to yellow or orange. The color of the peel results from a combination of pigments including chlorophyll, carotenoids, anthocyanins, and lycopene. However, this external color change is a poor indicator of maturity; it is a function of climate more than fruit maturity. Fruit growth rate is primarily a function of temperature during each developmental stage with the highest mean temperatures providing the fastest fruit growth rates. Tree vigor also has a pronounced effect on fruit color. Vigorously growing trees produce more poorly colored fruit than slow-growing trees. Citrus matures slowly and once harvested, it does not continue to ripen. The maturity of the fruit is determined by gradual changes in juice content, and sugar and acid levels. As the fruit matures, the acid content decreases and sugar content increases. Most citrus fruits can be left on the tree without becoming overripe, though they do become senescent. Citrus fruit has two abscission zones, one at the base of the pedicel and other at the base of the ovary. Two major kinds of abscission may be discerned during fruit development. Fruitlet abscission is a self-thinning mechanism that adjusts the number of fruits to the tree's bearing potential (Goldschmidt and Monselise 1978). On the other hand, the shedding of the mature fruit may be regarded as a mechanism of seed dispersal (Spiegel-Roy and Goldschmidt 1996).

4.5 Polyembryony

The formation of multiple embryos is quite common in many citrus cultivars. They may be the result of multiple zygotic embryos, produced by the fission of one fertilized egg or from two or more functional embryo sacs in a single ovule (Bacchi 1943; Cameron and Garber 1968). However, the predominant cause of multiple embryo formation is nucellar embryony, the development of vegetative embryos from the nucellus. These embryos are the outgrowths of the nucellus and develop asexually by mitotic division of the cells of the nucellus. As the male gamete does not contribute to their formation, they are the product of vegetative reproduction having a genetic constitution identical to that of the female (seed) parent (Nageswara et al. 2008). This asexual reproduction is an important characteristic in citrus and has very important consequences for the evolution, breeding, and culture of citrus fruits (Frost and Soost 1968). Anatomical studies of open and controlled pollinations have indicated that adventive embryos are initiated autonomously and develop with or without pollination (Wakana and Uemoto 1987). However, pollination is essential for the stimulation of nucellar embryo development as they fail to develop without endosperm development. Early degeneration of endosperm also results in very poor seed development and, eventually, in poor development of adventive embryos (Wakana and Uemoto 1987). These nucellar embryos grow more rapidly than the zygotic embryo within the seed. One possible reason for this may be that the zygotic embryo is located unfavorably in the apex of the embryo sac (Toxopeus 1936; Iwamasa et al. 1970), receives fewer nutrients, and is more subject to crowding pressure; in addition, zygotic embryos derived from self-pollination in normally heterozygous cultivars could be associated with inbreeding depression expressed in zygotic embryos (Toxopeus 1936). If polyembryonic genotypes are used in crossing as female parent, several nucellar seedlings similar to the mother plant and very few or no hybrids are produced. This characteristic allows the selection of improved mutants, which have better yield efficiency and fruit quality than the parent. The satsuma varieties ‘Mihu’ and ‘Okitsu’ are nucellar selections of ‘Miyagawa’. The plants arising from nucellar seedlings are generally free of viruses. Citrus are almost universally propagated by budding onto seedling rootstocks (Xiang and Roose 1988). Uniformity of the rootstock genotypes is essential for reliable performance following budding and orchard establishment. Nucellar embryony allows fixing the genotype of a superior variety, and hence seed can be produced for many generations without loss of vigor or genotype segregation, circumventing any need to produce hybrid seeds for rootstock production (Garcia et al. 1999).

5 Breeding

Citrus (most frequently $2n = 2x = 18$, though higher ploidy levels occur spontaneously and have deliberately been produced) is vegetatively propagated. Selection of new citrus and related cultivars has been occurring for many years by selection of superior phenotypes from the wild for cultivation. However, systematic, mission-oriented breeding programs first began in Florida in 1893 with Swingle and Webber (Davies and Albrigo 1994). Since then, numerous programs have been developed worldwide with a variety of objectives. Due to its heterozygous nature, sexual hybridization to create new genotypes results in substantial variation of the characters in the progeny as they produce widely variant sexually derived progeny. Nucellar embryos, on the other hand, give rise to genetically and phenotypically uniform progeny. A long period of juvenility is characteristic of citrus seedlings and is evidenced by the presence of thorns, vigorous upright growth, delay in fruiting, and alternate bearing. It takes 5 or more years for the first flowering to occur in citrus seedlings. This long juvenile period of seedlings makes citrus breeding not only a difficult but a costly and land-intensive proposition.

There is a lack of knowledge regarding genetic mechanisms controlling the inheritance of agriculturally important traits. Only a few important traits show single gene inheritance (Furr 1969; Gmitter et al. 1992). Conventional hybridization has given rise to a few new citrus cultivars and rootstocks (Soost and Cameron 1975; Cameron and Soost 1984; Gmitter et al. 1992). Use of citrus cultivars and selections that give rise to only apomictic seeds as seed parents leads to the production of few or no hybrids as the apomictic seeds contain only asexual embryos. Due to inbreeding depression, crosses between closely related lines will produce primarily weak zygotic seedlings. Although it takes only minutes to effect a pollination, the difficult nature of citrus breeding lies in the elimination of undesirable hybrids and the evaluation of selections (Sykes 1987). Despite the fact that citrus breeding is very challenging, different breeding programs throughout the world have made significant progress in the application of conventional and modern approaches to genetic improvement and cultivar development. Important breeding goals exist in citrus with respect to both scions and rootstocks (Cameron and Frost 1968).

Conventional methods of breeding scion and rootstock cultivars are generally based on controlled crosses. To combine desirable traits from different selections, cultivars, or species in hybrid progeny, cross-pollination is carried out. After the hybrid fruits have matured, the seeds are extracted from the fruit and planted in the greenhouse. Once the seedlings have attained sufficient size, they are either grafted onto rootstocks or directly planted in the field for evaluation of their performance. The hybrids are evaluated for disease and pest resistance, stress tolerance, and overall growth characteristics during the juvenile period (Davies and Albrigo 1994), and subsequently for fruit

characteristics of scions or rootstock traits of interest. Mutation breeding programs have also been established for the genetic improvement of citrus. Mutations have been induced by gamma rays and chemicals and the mutants analyzed for the desired traits (Hensz 1977; Hearn 1984; Deng and Zhang 1988; Deng et al. 1993; Gulsen et al. 2007).

5.1 Scion

The scion breeding programs are mainly aimed at improving the fruit color, size, shape, flavor, and yield, as well as low seed content, easy peeling, and disease resistance. The main breeding aims for scion cultivars vary with species and localities. The first step in scion breeding involves selection of parental types with favorable heritable characteristics/traits. Often those seed parents are selected that produce only zygotic progeny (Soost and Cameron 1975). Hence monoembryonic parents are preferred for scion breeding. It is traditionally achieved by controlled crossing. When there is a need to combine desirable traits/characteristics from different species, cross-pollination is carried out. The hybrid fruits are allowed to grow and harvested at maturity. The seeds are extracted from the fruit and planted in the greenhouse initially. Once the seedlings have attained sufficient size, they are then planted in the field where they are grown to fruiting and evaluated for their fruit characteristics. Once the desired scion hybrid is selected, it is budded onto different rootstocks to further evaluate its performance. The hybrids are also tested for biotic and abiotic stress tolerance, and their overall growth characteristics are also monitored. Multiyear and multilocation field trials are conducted to evaluate their performance with major emphasis on the fruit quality (size, shape, exterior rind characters, peel thickness, pulp characteristics, and seediness), and yield.

Most citrus cultivars have resulted from natural hybridization of well-adapted native cultivars, spontaneous mutation, bud sport mutations, or apomictic seedling mutants (Hodgson 1967). Many of the widely grown scion cultivar groups, such as sweet orange, grapefruit, lemon, and various clonal selections of certain mandarin cultivars such as ‘Satsuma’ and ‘Clementine’, originated as either bud sport mutations or apomictic seedling mutants. No cultivars of these have ever originated as sexually derived seedlings (Gmitter et al. 1992). They are not amenable to sexual hybridization as a genetic improvement strategy. Hence, selection of useful variations following induction via mutagenic treatment of seeds and axillary buds, from spontaneously occurring nucellar seed or bud mutations, or somaclonal variation (Grosser et al. 2003, 2007), have been the only effective approaches to cultivar development in these cultivar groups. The irradiated seeds of ‘Hudson’ grapefruit gave rise to ‘Star Ruby’ grapefruit, which had deep red flesh and reduced seediness (Hensz 1977). A low–seeded, grapefruit-like hybrid (USDA 77-19) was developed by

USDA citrus breeding program by irradiating the hybrid 'USDA 75-8' selected from a population of 'Pearl' tangelo × grapefruit (Chaparro 2003). A special objective of increasing significance is the breeding of grapefruit cultivars with low levels of acidity and less bitterness (Spiegel-Roy and Goldschmidt 1996). Trees propagated from irradiated buds of 'Foster' grapefruit gave a mutation in the acid metabolic pathway that resulted in low acid production and early fruit maturity (Yen 1987). Irradiated buds of 'Kutdiken' lemon have given rise to plants showing variations for fruit maturation time, flowering, branching habit, and thorniness (Gulsen et al. 2007). Seedlessness is a prime requirement for fresh fruit. Mutation breeding by irradiation of seeds and/or axillary buds has also given rise to seedless clones of normally seedy 'Pineapple' orange, 'Duncan', and 'Foster' grapefruit (Hearn 1984, 1985), 'Monreal' Clementine mandarin (Russo et al. 1981), 'Eureka' lemon (Spiegel-Roy et al. 1985, 1990; Miller et al. 2003), and 'Kutdiken' lemon (Gulsen et al. 2007). A seedless 'Minneola' tangelo has also been produced by mutation breeding (Spiegel-Roy and Vardi 1989). Irradiation of seeds of 'Jincheng' sweet orange has given rise to a seedless clone 'Zhongyu No 7' (Deng 2003). Irradiation of axillary buds of 'Kutdiken' lemon has also been used to obtain mutants resistant to *mal secco* (Gulsen et al. 2007). A seedless mandarin 'Tango' has been produced by irradiation of buds of W. Murcott (Roose and Williams 2006a). 'Monreal verde' is an almost seedless variety obtained by irradiating budwood of 'Monreal' Clementine (Nicotra 2001).

Mandarins are relatively easy to breed by crossing parents with good traits and selecting superior progeny. 'Fairchild' mandarin, a hybrid of 'Clementine' mandarin and 'Orlando' tangelo, has proven to be particularly well-suited to the California and Arizona deserts where it provides an early season fruit for the market (Furr 1964). 'Encore', a late-ripening variety, which originated from a cross between 'King' and 'Willowleaf' mandarins, was introduced in 1965 (Cameron et al. 1965). 'Fallglo' tangerine is a hybrid of 'Bower' and 'Temple' and was released in 1987 (Hearn 1987). USDA 88-2, a cross between 'Lee' and 'Nova' mandarins, is very juicy and easy-to-peel early season seedless mandarin with small to medium size fruit and is under evaluation for commercial potential. USDA 88-3 is a cross between 'Robinson' and 'Lee' mandarins and an early season mandarin; however, it is not considered for commercial purposes as the fruit is very seedy. 'Tacle' and 'Clara' seedless mandarins were obtained by crossing 'Monreal' Clementine and 'Tarocco' orange (Nicotra 2001). 'Gold Nugget', a seedless late-maturing diploid hybrid between 'Wilking' and 'Kincy', has been released (Roose et al. 2000; Roose and Williams 2003). 'Daisy', a hybrid between 'Fortune' and 'Fremont' mandarins, produces a medium-large, mid-season mandarin with an attractive dark orange rind with moderate peelability and sections. 'Camel' mandarin was a seedless hybrid of 'Nules' Clementine and 'Willowleaf' mandarin (Nicotra 2001). 'Shasta Gold®'or 'TDE2' (a late maturing), Tahoe Gold®' or 'TDE3' (mid-season maturing), and 'Yosemite Gold®'or 'TDE4' (mid-late season maturing) are mandarin

hybrids of ('Temple' tangor × 4*n* 'Dancy' mandarin) and 'Encore' mandarin that combine large fruit size, attractive deep orange rind color, rich fruit flavor, and the virtual absence of seeds (Roose and Williams 2006b). 'Winola' was a spontaneous triploid hybrid selected among a population of diploid hybrids between 'Wilking' mandarin and 'Minneola' tangelo (Nicotra 2001). 'Primosole', 'Simeto', 'Desiderio', 'Bellezza', 'Sirio', and 'Cami' are some other examples of seedless hybrids released (Nicotra 2001).

As large areas of citrus have low winter temperatures, several breeding programs also aim to incorporate cold hardiness (Spiegel-Roy and Goldschmidt 1996). Limequats (*Fortunella* × *C. aurantifolia*), citranges (*C. sinensis* × *P. trifoliata*), and citrumelos (*C. paradisi* × *P. trifoliata*) are some of the examples of intergeneric hybrids produced by controlled pollination with the objective of transferring cold tolerance to citrus. Although the cold tolerance was achieved, none of these intergeneric hybrids gained commercial acceptance as scion cultivars due to the transmission of negative fruit quality attributes along with cold tolerance (Gmitter et al. 1992). Reduced tree size without reduction in yield per unit volume is highly desirable as picking costs increase. Unusually early- or late-maturing new forms are always of interest, since they may fill a need in a pattern of production or marketing (Cameron and Frost 1968). Disease and pest resistance of the scion cultivars is desirable but difficult to achieve as gene sources are either not available or distantly related to the scion cultivar that recovery of acceptable cultivars is unlikely. Season of ripening, storage life, and adaptability to specific environments often determine the success or failure of new cultivars (Soost and Roose 1996).

Clonal selection can be used to isolate superior bud source strains of established cultivars and to eliminate propagation of undesirable budlines (Shamel 1943). Clonal selection within cultivar groups ('budline selection') has been useful for the development of improved strains of cultivar groups like *Satsuma mandarin* and navel sweet orange. Sexual hybridization will continue to receive attention for mandarin cultivar development because of increased numbers of improved monoembryonic breeding parents available for hybridization. Interspecific hybrids between mandarin-sweet orange (tangors) and mandarin-grapefruit (tangelos) have been developed by breeding programs (Gmitter et al. 1992).

5.2 Rootstock

The need for dependable new rootstocks is of primary concern as they affect all aspects of fruit quality. However, choice of rootstock is not usually based on fruit quality considerations alone; disease tolerance, soil type, and effects on yield are more often overriding considerations (Bevington 1987). Reduction of tree size without affecting yield or scion health is desirable (Soost and Roose

1996). The major objectives of rootstock breeding are aimed to control the tree size and to improve resistance and tolerance to biotic and environmental stresses such as citrus blight, CTV, *Phytophthora*, CVC, nematodes, cold, drought, salinity, and flooding. Rapid growth and lack of branching are desirable characters for convenient and economical nursery production of rootstock seedlings (Soost and Roose 1996).

Like scion breeding, rootstock breeding also involves controlled crossing. However, in rootstock breeding, highly polyembryonic species are selected as parents or at least one parent which is polyembryonic. Potential rootstocks showing favorable qualities are planted. Extensive multiyear and multilocation field trials are conducted to evaluate all aspects of rootstock performance. They are screened for resistance to various soil-borne diseases or stresses, compatibility with various scion cultivars, and effects on fruit quality and yield. Rootstock candidates are also screened for the seediness of fruit and the uniformity of seedling populations, as most citrus rootstocks are propagated from apomictic seeds.

Many commonly used rootstocks have not been products of planned breeding programs; rather they have been selected over time through grower experience. These include selections of various citrus species such as sour orange (*C. aurantium*), rough lemon (*C. limon*), Cleopatra mandarin (*C. reticulata*), Rangpur lime (*Citrus* × *limonia* Osbeck), and numerous others. Sexual hybridization has been used to produce genetically improved combinations of rootstocks. Carrizo and Troyer citranges (*C. sinensis* × *P. trifoliata*) and Swingle citrumelo (*C. paradisi* × *P. trifoliata*) rootstocks were selected from intergeneric hybrid progeny and were found to have *Phytophthora*, virus, and nematode tolerance inherited from *P. trifoliata*. Many other sexual hybrids have been made in efforts to exploit available genetic diversity for rootstock improvement in breeding programs around the world. A hybrid 'US-852' obtained from sexual hybridization of *C. reticulata* and *P. trifoliata* was found to exhibit outstanding effects on sweet orange fruit yield, producing fruit with high soluble solids on medium-size trees (Bowman et al. 1999). The IVIA in Spain has released four rootstocks, of which two (Forner Alcaide 5 and Forner Alcaide 13) were obtained by sexual hybridization between Cleopatra mandarin and *P. trifoliata*, one (Forner Alcaide 418) of Troyer citrange and *P. trifoliata*, and one (Forner Alcaide 517) of King mandarin and *P. trifoliata*. All the four rootstocks were resistant or tolerant to CTV and salinity. Forner Alcaide 5 was also found to be resistant to the citrus nematode and had good tolerance to calcareous soils and flooding. Forner Alcaide 418 was a dwarfing rootstock with good tolerance to calcareous soils and induced large fruits in the scion cultivar. Forner Alcaide 517 was also a dwarfing rootstock and had good tolerance to calcareous soils (Nicotra 2001; Forner et al. 2003). 'X639', a hybrid between 'Cleopatra' mandarin and *P. trifoliata*, has proved to be an excellent rootstock for lemons and mandarins; however, it is not resistant to nematodes and root pathogens (Miller et al. 2003).

6 Biotechnology

Traditional breeding approaches via sexual hybridization have not been useful for the genetic improvement of most of the citrus cultivars. Although spontaneous or induced mutations tend to be random and are not directed toward a specific target, they have resulted in varietal improvements. Application of the biotechnological sciences such as plant cell and tissue culture, genetic engineering, and genomics has the potential to unlock an entirely new round of genetic improvements for citrus crops. The genetic progress is impeded by barriers to sexual hybridization and the hybrid nature of important cultivated species. The scarcity of genetic information can be addressed and potentially mitigated by these biotechnological techniques (Gmitter et al. 1992).

6.1 Regeneration and Micropropagation

The direction for the genetic improvement of citrus is greatly impacted by the advances in plant cell and tissue culture. The amenability of citrus to be regenerated via organogenesis and somatic embryogenesis is the fundamental basis that makes possible much of the potential for these genetic advances. Plant regeneration systems are potentially useful for obtaining genetic change through cell transformation or mutagenesis. Organogenesis has been induced in vitro from various explants such as shoot meristems of seedling and mature trees (Barlass and Skene 1986; Omura and Hidaka 1992; Kotsias and Roussos 2001), nodal explants (Al-Khayri and Al-Bahrany 2001), stem internodes (Moore 1986), leaf sections (Chaturvedi and Mitra 1974; Huang et al. 2002), and root tissues (Sauton et al. 1982). In vitro culture of excised, fully developed embryos (Maheshwari and Rangaswamy 1958), early heart-shaped embryos (Rangan et al. 1969), globular embryos within undeveloped ovules of mature fruits (Starrantino and Russo 1980; Carimi et al. 1998), and immature embryos (Cavalcante-Alves et al. 2003) has also been used to recover plants. In vitro seedling explants have also been used for multiple shoot formation and/or regeneration (Normah et al. 1997; Yang et al. 2006). Regeneration has also been achieved by culturing thin sections of mature stem segments (Kobayashi et al. 2003; Soneji et al. 2007a)

Somatic embryogenesis is particularly attractive in citrus because many genotypes have the capacity for nucellar embryony (Soost and Roose 1996). Somatic embryogenesis has been induced directly in cultured nucelli (Rangan et al. 1969) and undeveloped ovules (Starrantino and Russo 1980; Gmitter and Moore 1986) or indirectly via callus formation (Kochba and Spiegel-Roy 1973; Button 1978; Koc et al. 1992; Tomaz et al. 2001; Kayim and Koc 2006). Embryogenesis has also been induced from endosperm-derived callus (Gmitter et al. 1990), juice vesicles (Nito and Iwamasa 1990), anthers (Chaturvedi and Sharma 1985;

Chiancone et al. 2006), styles (Carimi et al. 1995; Carimi et al. 1998; Calovic et al. 2003; D'Onghia et al. 2003), and pistil thin cell layers (Carimi et al. 1999).

6.2 Somaclonal Variation

The identification of valuable somaclonal variants holds great promise for cultivar improvement especially for the citrus species that are difficult to manipulate by sexual hybridization (Gmitter et al. 1992). Somaclonal variation has been observed in citrus plants regenerated from nucellar callus of monoembryonic 'Clementine' mandarin (Navarro et al. 1985). It is being exploited to identify sweet orange clones with improved traits such as fruit quality improvements across an extended season of maturity (Grosser et al. 2003). Preliminary observations of in vitro derived nucellar budlines of 'Navelate' sweet orange indicated that performance of two budlines may be superior to the others in terms of fruit quality and yield (Starrantino et al. 1990). Somaclones of 'Hamlin' and 'Valencia' have been obtained via regeneration of adventitious shoot buds, regeneration of secondary embryogenic callus via somatic embryogenesis, and/or regeneration from protoplast via somatic embryogenesis. Of these, early- and late-maturing somaclones, somaclones with fresh market potential, as well as somaclones with elevated soluble solids of 'Valencia' and 'Hamlin' with improved color are under field trial (Grosser et al. 2003). Callus lines have been selected for salt tolerance (Kochba et al. 1982; Spiegel-Roy and Ben-Hayyim 1985) and regenerated into plantlets. These plantlets lacked internodes and hence could not be propagated further (Ben-Hayyim and Goffer 1989). *C. limon* embryogenic culture lines resistant to 'mal secco' toxin were selected. These lines produced somatic embryos, which retained resistance to the toxin (Nadel and Spiegel-Roy 1987). 'Femminello' lemon somaclones have also been evaluated for tolerance to mal secco by artificial inoculation (Gentile et al. 2000). However, the field resistance in the mature plants has yet to be reported.

6.3 Ploidy Manipulation

Euploidy in citrus is represented by monoploids, diploids, triploids, tetraploids, pentaploids, hexaploids, and octaploids. Polyploid plants may offer considerable potential for cultivar improvement through exploitation of their horticulturally useful characteristics and as parents in breeding programs, particularly the triploid and tetraploid lines (Lee 1988).

Production of triploid hybrids is the most promising approach to obtain cultivars that do not produce seeds even with substantial cross-pollination (Navarro et al. 2004). Recovery of citrus sexual triploid hybrids ($3x = 27$) has been reported from $2n \times 4n$ (Cameron and Soost 1969), $4n \times 2n$ (Cameron

and Burnett 1978), and $2n \times 2n$ (Esen and Soost 1971) crosses. When the pistillate parent was used as tetraploid, 85% triploid progeny were recovered (Cameron and Burnett 1978). This high triploid recovery arises from normal sexual fertilization of the diploid female gamete with a haploid male gamete (Cameron and Burnett 1978). 'Shasta Gold®'or 'TDE2' (a late maturing), Tahoe Gold®'or 'TDE3' (mid-season maturing), and Yosemite Gold®'or 'TDE4' (mid-late season maturing) are triploid mandarin hybrids of tetraploid female parent ('Temple' tangor $\times$ 4*n* 'Dancy' mandarin) and diploid male parent 'Encore' mandarin that combine large fruit size, attractive deep orange rind color, rich fruit flavor, and the virtual absence of seeds (Roose and Williams 2006b). Compared to $4n \times 2n$ crosses, $2n \times 4n$ crosses gave rise to only 9% of triploid hybrids (Cameron and Soost 1969). 'Tacle' and 'Clara' seedless triploid mandarins were obtained by crossing diploid female parent 'Monreal' Clementine and tetraploid male parent 'Tarocco' orange, while 'Camel' mandarin was a triploid hybrid of diploid female parent 'Nules' Clementine and tetraploid male parent 'Willowleaf' mandarin (Nicotra 2001; Recupero and Tribulato 2003). 'Reale' isolated from a cross of 'Monreal' Clementine and *Fortunella hindisii* (4*x*) is interesting as an ornamental potted tree which is everblooming and early fruiting (Russo et al. 2003). In triploid progenies, the characteristics of many genes (fruit size, flavor, etc.) that are key factors for breeding programs seem to be strongly influenced by the tetraploid parent (Russo et al. 2004). In $2n \times 2n$ crosses, the triploid embryos are originated by the fertilization of an unreduced diploid female gamete with a normal reduced haploid male gamete. Breeding triploids from $2n \times 2n$ crosses eliminates the need for tetraploid parents, thereby overcoming the problem of aneuploidy which may result from irregular meiosis in the tetraploids (Esen and Soost 1971; Geraci et al. 1975). It is very difficult to regenerate plants by conventional methods from seeds with triploid embryos as these embryos are generally small in size, underdeveloped, or aborted. Embryo rescue has enabled breeders to rescue such genetically valuable embryos that otherwise would not develop into whole plants (Starrantino and Recupero 1982). Embryo rescue and culture in vitro are necessary because embryos that arise when diploid seed parents are crossed with tetraploid pollen sources do not undergo normal development. In normal ($2n \times 2n$) crosses, the ratio of the endosperm chromosome set of maternal–paternal must be 2:1 for normal development of endosperm following hybridization. However, in $2n \times 4n$ and $4n \times 2n$ crosses, the ratio of the endosperm chromosome set of maternal–paternal is 2:2 and 4:1, respectively, leading to degeneration of endosperm. The imbalance between the embryo and endosperm ploidy (3:4 or 3:5) rendered seeds from such crossings incapable of germinating in vivo. Triploids have also been regenerated by in vitro culturing of hybrid endosperm (Gmitter et al. 1990). However, this method has not been adapted as a breeding strategy because it is species and cultivar dependent, and is far less efficient than creating triploid offspring by interploid hybridization. Regenerants of anther culture of *C. clementina* were found to be triploids

(Germana et al. 2005). Triploid citrus hybrids have also been obtained by electrofusion of a gynogenetic haploid cell line of Clementine and several diploid cultivars (Ollitrault et al. 2000)

Tetraploid progenies have arisen from all crosses of diploid and tetraploid parentage using monoembryonic seed parents. This may be due to nondisjunction leading to the doubling of chromosome number of one of the parents during cell division. Incorporation of colchicine in standard tissue culture media has made it possible to recover tetraploid plants of elite diploid selections or cultivars (Gmitter and Ling 1991; Gmitter et al. 1991). Tetraploids have also been produced by somatic hybridization (Grosser et al. 1998). The main uses of citrus tetraploids have been either as rootstock candidates combining complementary parents, or as parents in breeding programs aimed at producing seedless triploids (Cameron and Soost 1969; Cameron and Burnett 1978; Grosser et al. 1998). Tetraploids generally grow slowly, are more compact in habit, and also produce less fruits as compared to diploids of the same cultivar (Soost and Roose 1996). Tetraploid clones of rootstock cultivars may find utilization as dwarfing rootstocks (Gmitter et al. 1992). A dwarfing effect by tetraploids in a rootstock trial comparing diploids with corresponding autotetraploids has also been reported (Lee 1988).

Anther culture may give the breeders and geneticists the ability to recover plants of reduced ploidy level providing them with another useful tool for citrus cultivar improvement and genetic studies (Gmitter et al. 1992). This would facilitate the recovery of useful recessive alleles or mutations by haploid plant regeneration. Citrus (Chen et al. 1980; Germana et al. 1991; Germana et al. 1994; Germana and Reforgiato 1997; Chiancone et al. 2006) and *Poncirus* (Hidaka et al. 1979) anthers have been cultured in attempts to produce haploid plants. Colchicine treatment can be used for doubling of chromosome number to yield homozygous diploids. Such plants would facilitate considerable advancement in the understanding of citrus genetics and would offer plant breeders much greater control over phenotypic expression (Lee 1988). However, anthers from sour orange (Hidaka et al. 1982), sweet orange (Hidaka 1984), and lime (Chaturvedi and Sharma 1985) yielded only diploid regenerants. Nuclear fusions that were sometimes observed in the routes of microspore development were suggested to be the cause of diploidy in regenerants (Hidaka and Omura 1989). The production of diploid plants by culturing anthers of tetraploid somatic hybrids may also provide breeders with greater access to these unique genetic combinations. In *C. natsudaidai*, haploid seedlings have been obtained by irradiation (Karasawa 1971). Haploid plants and embryogenic calli of Clementine have been obtained after in situ parthenogenesis induced by irradiated pollen (Ollitrault et al. 1996). Haploid plantlet regeneration through gynogenesis of Clementine has been induced by in vitro pollination with pollen from a triploid plant (Germana and Chiancone 2001). Tri-haploids have been obtained by regeneration of anthers of *C. clementina* (Germana et al. 2005).

6.4 Somatic Hybridization

Somatic hybridization allows production of somatic hybrids that incorporate genomes of the two parents without recombination, thus avoiding the problem of the high heterozygosity in citrus (Navarro et al. 2004). In citrus, this technology has been extensively used and has many important implications (Grosser et al. 2000). The first successful protoplast isolations were reported as early as 1982 (Vardi et al. 1982), and the first citrus somatic hybrid was obtained between *C. sinensis* and *P. trifoliata* (Ohgawara et al. 1985). These results allowed the establishment of citrus breeding programs in several countries, both for scion and rootstock improvement (Grosser and Gmitter 1990). Somatic hybridization has provided a means of producing heterozygous tetraploid hybrids, which have incorporated complementary traits from donor parents. It has made production of hybrids from sexually incompatible or difficult to hybridize citrus relatives that possess valuable attributes possible, thus broadening the germplasm base available for rootstock improvement. Somatic hybrids have been developed, at the Citrus Research and Education Center, Florida, USA, from more than 150 parental combinations and are now in field trials to determine their potential in scion and rootstock improvement (Grosser et al. 2000). With the cost of production increasing over time, there is greater emphasis on reducing the tree stature to make orchard management and crop harvesting more efficient and also to bring young trees into economically valuable production earlier. In some cases, the somatic hybrids have combined desirable disease resistance and stress tolerance traits, and confer varying degrees of tree size control and precocity as well.

Somatic hybridization has also been used to create new tetraploid somatic hybrids that combine elite diploid scion material to be used as tetraploid breeding parents in interploid hybridization schemes to develop seedless and easy-to-peel new mandarin varieties (Grosser et al. 1998). Another approach to seedlessness is the transfer of cytoplasmic male sterility from Satsuma oranges to other elite but seedy scions via cybridization. This approach has the potential to make existing popular cultivars seedless, without altering the cultivar integrity in any other way. Creation of triploid citrus hybrids by electrofusion of haploid and diploid protoplasts is also promising (Ollitrault et al. 2000). Progress has also been made in the development of improved acid fruits (lemons and limes) and ornamental citrus, through somatic hybridization and the subsequent use of the hybrids in sexual crosses.

6.5 Transformation

Genetic transformation may provide an efficient alternative for citrus improvement, opening the way for the introduction of specific traits into known

genotypes without altering their elite genetic background. The systems of transformation that have been used for genetic engineering experiments are dependent upon the fundamental abilities of in vitro regeneration. Genetic engineering has been applied to an increasing number of traits for citrus improvement. Gene constructs have been created for various types of CTV-derived genes (Gutierrez et al. 1997; Dominguez et al. 2000; Ghorbel et al. 2001; Fagoaga et al. 2006) and other genes from the naturally resistant *Poncirus trifoliata* (Soneji et al. 2007b), and have been inserted into citrus genomes in efforts to induce resistance to the CTV virus. A citrus blight associated gene has also been introduced into Carrizo citrange (Kayim et al. 2004). Genetic transformation and regeneration of mature tissues of citrus, which could bypass the juvenile phase, has also been attempted (Cervera et al. 1998). A gene for tolerance to salinity (HAL2) from yeast has also been introduced into citrus (Cervera et al. 2000). Genes that regulate vegetative and other behavior in *Arabidopsis* have been engineered into citrus resulting in altered growth habits and greatly reduced juvenility (Pena et al. 2001). Genes involved in metabolic pathway regulation, such as those in the flavonoid pathway, have also been introduced in citrus (Costa et al. 2002). The growing interest in manipulating carotenoid biosynthesis in plants is mainly related to human nutrition as precursors of vitamin A and natural antioxidants. Also, this kind of pathway manipulation holds promise of altering color and flavor development in citrus. *CS-ACS1* gene that controls the ethylene biosynthesis has also been introduced into citrus (Wong et al. 2001), and the transgenic lines that produced higher level of antisense ACS RNA repressed the increase in ACC content following chilling treatment. Attempts have also been made to introduce juice quality related pectin methylesterase gene into citrus (Guo et al. 2005). This exploitation of enzymes associated with 'cloud separation' may also offer a great promise of targeted trait modification and improvement of the responsible genes by genetic manipulation. The cDNA of the *Xa21*, a *Xanthomonas* resistance gene, has been introduced into citrus via protoplast cotransformation (Omar and Grosser, 2007). Study is underway to challenge these transgenics with canker at a state Division of Plant Industry quarantine facility in Gainesville, Florida, USA, to determine whether this gene has any potential in the improvement of citrus cultivars for canker resistance.

6.6 Genomics

Few studies have been carried out to understand the genetics of citrus. There is a lack of knowledge and understanding of the genetic mechanisms that control important traits such as disease resistance, cold tolerance, juvenility/maturity, and aspects of fruit ripening process (Gmitter et al. 1992). The entire field of citrus biology and genetics can be revolutionized by expanding the potential

capabilities of genomics and bioinformatics to cultivar improvement through precise and targeted manipulations of the genome.

The rapid development of molecular marker technologies has made it possible to investigate gene expression, and has helped in construction and integration of genetic and physical maps of the economically important traits such as CTV resistance (Gmitter et al. 1996; Deng et al. 1997), nematode resistance (Ling et al. 2000), fruit acidity (Fang et al. 1997), and dwarfing (Cheng and Roose 1995). These genetic maps may provide the basis for early screening procedures, thus permitting breeders to make initial selection among very young progeny based on the phenotype predicted by their genotype at molecular loci known to cosegregate with a particular phenotype (Durham et al. 1992). It would be possible to improve the efficiency of conventional plant breeding by mapping the desired genes and carrying out selection not directly on the trait of interest but on molecular markers linked to genes influencing that trait.

Molecular marker technologies also provide tools to tag the genes of known phenotypes by developing localized molecular linkage maps. These are very essential for map-based cloning (MBC) approach and marker-assisted selection (MAS) breeding programs (Recupero et al. 2000; Asins 2002). Dominant trifoliate leaf, a morphological trait, was considered to be the earliest MAS marker and was used to distinguish zygotic hybrids from nucellar seedlings, but morphological characteristics cannot be used with varieties without such distinct traits. Hence, MAS is usually carried out with the help of biochemical and/or DNA-based markers. Markers such as isozymes, RAPDs, and EST-SSRs have been used for the identification of hybrids (Soost and William 1980; Nageswara Rao et al. 2008). DNA-based molecular markers may be used to select rootstocks that may contain many of the desired resistances to CTV (Gmitter et al. 1996), nematode (Ling et al. 2000), *Phytophthora,* etc. This will prove to be highly cost-efficient as compared to traditional greenhouse or field-screening approaches using inoculation, thus making the multitrait selection possible in a substantially shorter period of time. As more key genes for critical traits are identified and tagged with easy to score molecular markers, MAS will improve the efficiency of the breeding process using traditional hybridization and selection strategies.

MBC is also called as positional cloning. It is another approach to isolate gene(s), without prior knowledge of gene product, using tools of comprehensive genetics, genomics, and bioinformatics. MBC of genes for CTV resistance from *P. trifoliata* has provided target gene sequences (Gmitter et al. 1998; Deng et al. 2001) to develop CTV-immune scion and rootstock varieties (Soneji et al. 2007b). Cloning of genes regulating cold-stress tolerance (Jia et al. 2004) and generalized disease resistance pathways have been accomplished. These sequences have been engineered into citrus plants to test their ability to modify plant performance against these two globally important limitations to citrus production.

Studies are underway to unravel the resistance to citrus canker expressed in kumquat, a closely related genus to citrus (Khalaf et al. 2007).

Molecular markers have also been widely applied on phylogenetic and taxonomic studies in citrus (Herrero et al. 1996; Fang and Roose 1997; Fang et al. 1997; Bret et al. 2001; Berkeley et al. 2006). Some efforts have also been made in the areas of resistance gene candidates (Deng et al. 2000; Deng and Gmitter 2003; Bernet et al. 2004), satellites (Fann et al. 2001), microsatellites (Kijas et al. 1995; Ahmad et al. 2003; Chen et al. 2008), variations from fragment restriction (Liou et al. 1996), methylation (Cai et al. 1996), and individual gene expressions (Moriguchi et al. 1998; Shimada et al. 2005). Integration of genetic linkage maps with the physical maps is also required for efficient localization and isolation of the genes, to study the organization and evolution of the genome, and as an initial step for efficient whole genome sequencing. Plans have been implemented for an international collaboration to develop integrated genetic and physical maps of the citrus genome, with an intention to lead to a full genomic sequence of citrus. Along with this will come the ability to understand genetic and metabolic control of all critical aspects of the traits of economic importance.

7 Conclusions

Citrus can be grown throughout the world in tropical and subtropical areas. It is vegetatively propagated. The great wealth of citrus types and cultivars of today reflects the vast natural breeding options within citrus, as well as effective intentional human intervention. Although citrus breeding is very challenging, breeding programs throughout the world are making significant progress in the application of conventional and modern approaches to genetic improvement. Advances in plant cell and tissue culture also have major impacts on genetic improvement of citrus. Somatic hybridization and recovery of monoploids, triploids, and tetraploids have expanded the range of germplasm available to citrus breeders. Triploid induction through endosperm culture, triploid hybrid embryo culture, or fusion of haploid with diploid protoplasts would enhance the possibility of developing triploid seedless cultivars. The benefits offered by monoploid culture or homozygotes to breeding programs and to the understanding of citrus genetics are incalculable. Although the prospects of in vitro culture to develop somatic hybrids are complicated by ploidy differences and the unpredictable fertility of wide hybrids, it has helped in overcoming sexual incompatibility among citrus species and cultivars

Most of the critical goals for scion improvement, such as resistance to diseases or changes in fruit quality attributes (color, flavor, peelability, nutrient content, etc.), are difficult to approach in any practical sense by conventional breeding strategies. It will be through genomic research that an understanding

of fundamental processes can be realized leading to the identification and cloning of candidate genes. Through genetic transformation, these candidate genes and their information will be exploited for the improvement of citrus. Genetic transformation promises to provide plant breeders with the ability to correct defects in existing elite cultivars by adding specific genes with little or no effect on other characters.

Selection of rootstocks is also critical. They vary greatly in their soil adaptability, relative susceptibility to diseases, viruses, interactions with scion, quality of fruit, size and vigor, and tolerance to soil-borne problems. Rootstock improvements will also be hastened and maximized through the application of new knowledge and tools developed from genomic technology. The knowledge and establishment of genomics and bioinformatics have provided efficient tools for tagging and cloning of important genes. It has also made the sequencing of the citrus genome plausible. Mapping and sequencing of a citrus genome would aid in elucidation of gene function, regulation, and expression. Advances in genomic science have a great impact in many respects, and will continue to provide new information and gene targets for manipulation.

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Pear Breeding

Manfred Fischer

1 Origin of Pears

The genus *Pyrus*, the pears, includes a wide range of species used partially as rootstocks but not or very rarely as human food. The genus *Pyrus* is a part of the family of Rosaceae with 34 chromosomes (2*n*). Judging by the supposed geographic origin, some wild species could be considered to be the ancestors of the cultivated pear: *P. pyraster* (L.), *P. elaeagrifolia* (Pallas), *P. spinosa* (Forssk.), *P. syriaca* (Bois.), *P.* × *nivalis* (Jacq.), *P. caucasica* (Fed.), etc. There is no doubt that the first one is the base of Central European varieties, but the lack of evidence makes it impossible to exactly determine the participation of other species in the evolution in terms of time and geography. *P. pyraster* (L.), the wild pear or wood-pear played an important role in the domestication of the cultivated pear—the European pear—*P. communis* (L.). Pears might have the same paleontological background as apples. The centres of genetic diversity of apples and pears are Central Asia; minor centres for pears are the Northern Caucasus, Minor Asia and the mountains of Northern Africa and Southern Europe. Asian pear, P. *pyrifolia* (Burm.) ('Nashi' pear, Japanese pear, Chinese pear and Sand pear) are cultivated throughout the whole of central and southern China, in the far east of Russia, Korea, Japan, South-East Asia, New Zealand, Australia and USA (California) and recently in southern parts of Europe as 'exotic' fruits. Most of the wild pears are diploid and crossable with cultivated pears and themselves. This can explain the relatively high variability of the genus *Pyrus* (Moore and Ballington 1992; Fischer and Weber 2005).

M. Fischer
IPK Gatersleben, Genbank Obst Dresden-Pillnitz (former affiliation), Söbrigener, Str. 15,
D - 01326, Dresden, Germany
e-mail: manfr.fischer@googlemail.com

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2 History

Information on the first pear cultivation commences from the Chinese Tsing and Han dynasties 2000 years ago. Primitive types were certainly cultivated earlier. Wild pears were eaten like wild apples by the Babylonian and Persian people, and in this way they were selected and propagated down the Silk Road before they were transported to Minor Asia and Europe. Nearly 1000 years ago, primitive pear growing was done in Europe with many different varieties. Belgium, North France and Italy developed as European centres of pear cultivation in the Middle Ages, where we find the oldest descriptions of pear varieties. Starting points for the development of the sources of modern varieties were *P. communis* for the European pears and *P. pyrifolia* as soon as *P. ussuriensis* for Asian pears. To the north in Europe, the Caucasian *P. caucasica* had some influence on the selection of more hardy primitive types of pear varieties (Janick 2004).

The domestication of Asian pears began in China about 2000 years ago. Today, more than 1000 varieties are known, which exist as many landraces. Even now we find wild populations with small fruits and a lot of grit cells in central China. An 'explosion' of pear breeding and growing occurred in Europe in 16th to 19th century. Especially in France and Belgium, many gardeners were working mostly as intelligent breeders, monks and nurserymen. Jean Baptiste van Mons (1765–1842) received more than 80,000 (!) seedlings for selection. All breeders were working more pragmatic with lots of opinions and practical knowledge. Scientific pear breeding started in the beginning of the 20th century in Belgium, France, USA and Germany. Today, the capacity of pear breeding is much smaller than apple breeding. However in Canada, USA, France, Italy, Czech Republic, Romania, Germany, Japan, China and Korea scientific institutions and private breeders have some remarkable successes in breeding new varieties. Recently, activities could be observed in China, USA and Switzerland for crossing Asian and European varieties for new desirable qualities (Petzold 1989; Fischer and Weber 2005).

3 Agricultural Aspects

The pear today is an alternative to apples. Asian and European pears were more important in the Middle Ages than they are now, but they are becoming more popular at present because of the dominance of apples in the market. Pear production is increasing in particular in countries with excellent climatic conditions, such as in Southern Europe, China, Argentina and Chile. In East Asia, main pear crops are Asian pears; in all other countries, there is a greater inclination to grow the European types of pears (Table 1). Nashi pears tend to be regarded as 'exotic' fruit in Europe. There is great deal of variability in

Table 1 Top 10 pear-producing countries (FAO 2004)

Country	1989–1992	1999–2002	%	Main cultivars
World	9870	16,606	+68	Ya Li, Bartlett, Tsu Li
China	2689	8679	+223	Ya Li, Tsu Li, Xuehua Li
Italy	892	896	+1	Abate Fetel, Bartlett, Conference
USA	841	861	+2	Anjou, Bartlett, Bosc
Spain	509	685	+34	Blanquilla, Conference
Argentina	265	546	+106	Bartlett, Packhams
Japan	437	388	−11	Kosui, Hosui, Nijisseiki
Turkey	417	369	−11	Santa Maria, Coscia, Ankara
South Korea	174	347	+99	Niitaka
South Africa	197	291	+48	Packhams, Bartlett, Forelle
France	345	269	−22	Bartlett, Guyot

size, shape, colour, flavour and ripening time. Use of fruits depended on the inherent quality and consistence. The eating quality is reached after different storage periods, possibly under controlled atmosphere. The fruits can be either canned or used for preparing juice and mixed drinks. Aromatic and sweet fruits can be processed to brandy under conservation of the typical pear flavour ("Williams Brandy").

The worldwide production of *P. communis* is dependent on relatively few cultivars. In a recent survey of major cultivars, 'Bartlett' ('Williams') is the major cultivar in many countries, followed by 'Beurre Bosc', 'Conference', 'Passa Crassane' and 'Doyenne du Comice'. The most important Nashi cultivars are 'Nijisseiki', 'Kosui', 'Chorujo' and 'Hosui'. Some cultivars in Asia are based on *P. ussuriensis* and *P. bretschneideri*, like 'Ya Li' and 'Tsu Li' of China. The major pear cultivars now are susceptible to some pests and diseases, especially *Pseudomonas syringae, Erwinia amylovora, Venturia pirina, Psylla* spp. and others. The present risks to production are significant, with the potential to cause yearly loss of marketable fruit, long-term problems on yield or the loss of trees. Many cultivars are deficient in production, fruit quality or storability. This is one of the reasons why the pears are not of economic importance comparable to apples. But germplasm accessions with better fruit features and tolerance or resistance to the most diseases and pests are known. More personnel and financial resources are needed to improve this situation. This would mean more work with genetic resources and an effective breeding of pears (Fischer and Weber 2005).

4 Breeding Efforts

The main breeding aims in the Middle Age to the beginning of the 20th century were

- excellent fruit quality for fresh consumption,
- pears for cooking,
- pears for drying, and
- vigorous trees.

The sowing of seeds after open pollination was the mostly used method of breeding. In this way, it was possible to receive a wide variability for the selection of new varieties. Many of enthusiastic people were integrated in testing of selected material in monasteries or fruit nurseries.

Controlled crossing (combination breeding) of selected parents begun in the middle of 19th century. A scientific base was given with the first results after evaluation of populations in different European and American institutions. The method of combination breeding by using heredity analyses is the main method of pear breeding in all the time. The most appreciated pear varieties have high degrees of heterozygosity. Cross-breeding leads to high degrees of variability in the progenies, thus making the selection of new genotypes using this traditional method easy.

There are numerous objectives of European pear genetic improvement, which are in large part dependent on the evolution of marketing and technological sectors. Previously, great emphasis was focussed on the improvement of agronomic and pomological characteristics, such as tree vigour, productivity and fruit appearance. Recently, however, there has been an increasing interest in the fruit-growing genetics and physiology, aimed at the development of production procedures capable of maintaining a correct balance of the fruit ecosystem. Thus, breeding objectives have been streamlined for tolerance or resistance to the most dangerous pests and diseases, adaptability to environmental factors, tree vigour control, extension of harvest period, fruit longevity and self-fertility. Particular attention deserves fruit quality, taste and nutritional characteristics (Petzold 1989; Brown 2003).

4.1 Breeding Objectives

The main breeding aims today are

- high and regular cropping,
- excellent fruit quality,
- winter pears with storage longevity,
- no grit cells,
- red or bicoloured fruits and
- fire blight resistance.

More detailed aims are different according to the region of breeding:

- compatibility with quince rootstocks
- attractiveness of the fruits
- drought tolerance (North America)

- calcium tolerance
- minimising the chilling requirement (South and North Africa, South America)
- winter frost tolerance (Northern China, Northern and East Europe, Canada)
- dwarf growing of tree (Europe)
- no alternate bearing
- virus tolerance or resistance
- resistance to pear psylla
- resistance to scab (*V. pirina*)
- resistance to pear decline
- resistance to *Pseudomonas*
- resistance to *Gymnosporangium sabinae*
- self-fertility

4.2 Genetics of Agronomic Traits

Knowledge of heritability of main characteristics is very important for the successful breeding work. The first steps are long-term evaluation of varieties in the field, in gene banks or in cropping plantations. The second steps have to be the testing of parents and populations. Many opinions exist today as summarised next by utilising the international literature and the results of German pear breeding. The following varieties, for example, are carrier of the genes for

- winter varieties—'Paris', 'Verté', 'Nordhäuser Winterforelle'
- summer varieties—'Bunte Juli', 'Trevoux', 'Clapp's Favourite', 'Bartlett', 'Starking', 'Santa Maria', 'Early Morettini'
- excellent fruit quality—'Doyenne du Comice' ('Vereinsdechantsbirne')
- poor fruit quality—'Countess de Paris'
- good fruit quality in combination with high yield—'Bosc's' ('Kaiser Alexander'), 'Dr. Jules Guyot', 'Präsident Drouard'
- very bad cropping—'Anjou', 'Packham's Triumph', 'Doyenne du Comice'
- fire blight resistance—'Seckel', 'Kieffer', 'Old Home', 'Harrow delight', 'Harrow Sweet', 'Harrow Gold', 'Alexander Lucas' (*P. communis*), 'Ya Li', 'Tzu Li' (*P.* × *bretschneideri*)
- scab resistance—'Bartlett' ('Williams'), 'Beurre Hardy' ('Gellert'), 'Kieffer', 'Dr. Jules Guyot'
- mildew resistance—'Doyenne du Comice', 'Winter Nelis'
- resistance to *Pseudomonas* blight—'Beurre Hardy', 'Forelle'
- tolerance to fruit rot—'Passa Crassana', 'Clapp's Favourite', 'Louise Bonne d'Avranches'
- cold hardiness—'Seckel', 'Doyenne du Comice', 'Erika', 'Delta'
- resistance to pear psylla—'Karamanka', 'Jerisbasma', 'Vodenjac', 'Monica'
- red colouration of fruits—'Nordhäuser Winterforelle', 'Rubia', 'Red Silk'
- dwarfness—'Armida', 'David', 'Abate Light'

The success of breeding for genetic resistance to diseases and pests is strongly dependent on the genetic value of varieties and species used in hybridisation. For a high level of resistance in new varieties, a cross-combination with resistant varieties is necessary (unfortunately, mostly they are of poor fruit quality, e.g., 'Sekel') or the use of different wild species for crossing, like

- *P. cordata*—scab resistance, mildew resistance
- *P. nivalis* –tolerance to pear decline
- *P. calleryana*—fire blight resistance
- *P. ussuriensis*—fire blight resistance, cold hardiness
- *P. elaeagrifolia*—generally fungal and bacterial resistance without fire blight

Using wild species is very problematic because of its extremely bad fruit quality and small fruit size. Four to five backcrosses are needed to evolve a type with acceptable fruit quality. Perhaps, biotechnology tools can help to solve the transmission of resistance genes into established varieties. Unfortunately in pear, heritability studies on resistance to diseases, and on many other important traits, are less advanced compared to apple (Zwet et al. 1974; Zwet and Keil 1979; Fischer and Mildenberger 1998; Hunter and Layne 1999; Andreies 2002; Bellini and Nin 2002).

Most pear varieties are susceptible to various viral, bacterial and fungal diseases. In addition, there are numerous animal pests, and crop protection measures must be taken. Protection against virus diseases is effected by utilising healthy planting materials. Virus-infected trees grow more slowly, the crop is smaller, the fruit quality, especially because of grit cell formation, is much worse and the compatibility between rootstock and grafted varieties is disturbed. Only streptomycin preparations are effective against the most important bacterial disease fire blight (*E. amylovora*); however, their use is disputed and not allowed in a number of pear-producing countries. Constant monitoring of the trees is necessary wherever this preparation is not usable so that infested young shoots can be detected in good time and eliminated. In damp and cold regions, bark blight (*Pseudomonas* spp.) can also appear in autumn. Cuprous agents help as a preventive measure against this. Dangerous fungus diseases affecting pears are scab (*V. pirina*) and mildew (*Podosphaera leucotricha*). Depending on climatic conditions, 6–20 fungicide sprayings are necessary in commercial cultivation to produce healthy fruits. When utilising organic production methods, only other plant sprays are applied. It is, therefore, impossible to obtain good fruit quality without plant protection measures (Brown 2003; Fischer and Weber 2005).

The best protection against these diseases is the utilisation of resistant varieties, with the aim to substantially reduce the amount of pesticides required. Resistant varieties help to solve many plant protection problems, specifically with regard to organic fruit growing. Therefore, the resistance breeding against fungi and bacterial diseases is one of the most important parts of pear breeding in the future.

There are numerous harmful animal pests. The most significant of which are pear psylla (*Psylla pyrisuga, P. piri, P. piricola*), codling moth *(Cydia pomonella*), various types of aphids (*Aphis* spp., *Dysaphis* spp., *Brachycaudus*

helchrysi, Myzus persicae, Hyalopterus pruni among others), the woolly aphid (*Eriosoma lanigerum*), scale insects (*Eulecanium corni, Lepidosaphis ulmi, Quadraspidiotus* spp. among others) and the red spider mite (*Panonychus ulmi*). Only insecticide and acaricide sprays help against these. Unfortunately, there are only a few varieties, land races or wild species with insect resistance or tolerance. That is why there are only few breeding possibilities to improve the resistance of new varieties in the future, and a success is not expected in the next time (Jones and Aldwinckle 1990; Weber 2001).

4.3 Biotechnology

4.3.1 In Vitro Culture

The contribution of in vitro techniques and molecular tools to genetic studies and breeding has advanced considerably in the recent past. Haploidisation via in situ parthenogenesis induced by irradiated pollen and in vitro rescue of the haploid plantlets has been developed (Chevreau et al. 1998; Bellini and Nin 2002). The aim is receiving double haploids (DH) for crossing with better knowledge on inheritance (Bouvier et al. 2002). This technique can be of interest especially for resistance breeding if it is possible to double heterozygous genes to 'homozygous' ones in DH plants. Techniques of adventitious bud regeneration from in vitro leaves have been developed for several genotypes of European and Asian pears. So far, applications of these techniques for the induction of somaclonal variation have been very limited (Chevreau et al. 1998; Dondini et al. 2002). Increased tolerance to fire blight has been obtained in somaclonal variants of some varieties (Chevreau and Skirvin 1992; Brown 2003).

4.3.2 Molecular Breeding

A major evolution of pear biotechnology is the development of *Agrobacterium tumefaciens* mediated transformation. About 10 genotypes were successfully transformed (Bell et al. 1999; Reynoird et al. 1999). Projects have started in several countries to express various transgenes in pear. Molecular markers developed now include isoenzymes, ALFPs, ISSRs, RAPDs and RFLPs. They have been used mostly for varietal identification of European and Asian pears. Markers have been developed recently for a few genes of interest, such as a black spot resistance gene and an ACC synthase gene (Chevreau et al. 1998; Oliveira et al. 1999) or the S-alleles for self-incompatibility of pears (Zuccherelli et al. 2002). But no genetic map of pear like for apples is yet available. About 20 genes have already been cloned mostly from Asian varieties. They are involved in fruit ripening or quality and self-incompatibility (Chevreau 2002; Lebedev et al. 2002; Tartarini and Sansavini 2003; Bell and Peterka 2004).

The use of biotechnical methodologies, the exploiting of somaclonal variation, and the setting up of early selection methods could make induced mutation techniques more reliable (gamma rays, chemical substances, etc.). It has intrinsic limitations, but it offers good prospects for further contributions to pear variety development and improvement as well (Hirata 1989; Masuda et al. 1997; Predieri 2002).

The pear consumer accepts improvements in a standard variety more easily than those that are completely new (Paprstein and Bouma 2000; Fischer and Weber 2005). In this way, spontaneous and induced mutations (in vitro or directly on plants) can be important for improvement of single traits of an already outstanding variety (Malnoy et al. 2000; Dondini et al. 2002; Monte-Corvo et al. 2002; Durel et al. 2004).

4.4 Cross-Combination Breeding in Germany

4.4.1 Breeding Aims and Selection

The aims of the German breeding program were excellent fruit quality, a good appearance and shape of fruit, early and abundant cropping and resistance to scab and fire blight. A special aim of selection was to find high-quality summer varieties and varieties with long storability. The crossing program involved only European varieties (*Pyrus communis*). For selection, following criteria were used: fruit quality (taste, colouring, no grit cells, consistency), ripening time, storability, growing capacity, susceptibility to scab and fire blight, yield and alternate bearing.

4.4.2 Results

The selection rate is listed in Table 2. Table 3 indicates the most successful cross-combinations with more than 10% elected seedlings in the populations. The

Table 2 Conclusion of selection rate in pears after three selection steps

	Seedlings	Elected clones	
Mother parent	*n*	*n*	%
Bunte Juli	751	26	3.46
Clapps Favourite	1342	33	2.45
Bartlett	587	22	3.74
Comice	1126	24	2.13
Madame Verté	451	1	0.09
Countess of Paris	1124	23	2.04
Nordhäuser Winterforelle	2261	54	2.38
Beurre Bosc	36	1	2.78
Gaishirtle	33	0	0
Desportes	37	1	2.70
Kongress	16	0	0

Table 3 Specific combination ability for good fruit quality

More than 10% selected clones received only from the following cross-combinations:
Clapps Favourite × Bunte Juli
Clapps Favourite × Nordhäuser Winterforelle
Nordhäuser Winterforelle × Baierschmidt
Nordhäuser Winterforelle × Madame Verté
Comice × Red Bartlett
Jules Guyot × Comice

Table 4 Breeding of winter varieties

	Percentage of seedlings ripened in		
Combination	Summer	Autumn	Winter
late × late (e.g., Nordh. Winterforelle × Countess of Paris)	5	87	8
late × early (e.g.. Comice × Trevoux)	42	54	4
early × early (e.g., Bunte Juli × Trevoux)	82	18	0

summary of cross-combinations to receive late ripening varieties is listed in Table 4.

The selection rate was never better than 4%, mostly only 1–2% without the cross-combinations listed in Table 3. Early ripeness dominated about late ripeness. It needs much more crossings for receiving winter varieties.

For all ripening groups, new varieties were selected. We found varieties for all production possibilities: for intensive and extensive cultivation methods for fresh market, for home gardening and for landscaping. All varieties are the result of improvement of the more or less known old pear varieties. The recommended and new pear varieties are

- summer (in time of 'Bunte Juli')—'Hermann', 'Isolda',
- autumn (in time of 'Bartlett')—'Gräfin Gepa',
- late autumn (in time of 'Bonne Louise')—'Armida', 'Manon',
- autumn to early winter (in time of 'Conference')—'Hortensia', 'Gerburg', 'Graf Dietrich', 'Thimo' and 'Elektra'.
- winter (in time of 'Alexander Lucas')—'David', 'Eckehard', 'Uta', 'Graf Wilhelm'.

The following conclusion listed the most important characteristics of the new German pears to explain the progress in breeding work. It was impossible to realise all the aims in one new variety. Every variety of necessity is a compromise.

- Yield, fruit size, growing capacity

The newly bred summer variety 'Isolda' has a stronger growth and is more yielding as 'Clapps Favourite'. 'Hermann' ripens much earlier but yield and fruit size are only average. But the colouration of 'Hermann' is excellent. The fruit size varies between 130 g ('Hermann') and 170 g.

Of the autumn varieties, 'Hortensia' is most productive, followed by 'Thimo', 'Elektra' and 'Graf Dietrich'. They are better yielding than 'Bartlett'. 'Armida' and 'Gräfin Gepa' are not too productive, but they have a very good fruit quality. 'Armida' is one of the prominent dwarfing varieties we know. 'Graf Dietrich', 'Gräfin Gepa' and 'Hortensia' grow stronger than 'Bartlett'. The fruit size of the autumn varieties varies between 170 and 250 g. 'Manon' has an excellent taste and good shape with mild susceptibility to fire blight, but it is susceptible to *Pseudomonas*.

Within the late varieties, 'Conference' is only surpassed by 'Eckehard' in yield, but all other varieties have a better fruit size and fruit quality than 'Conference'. 'Uta' and 'David' are very dwarf as regards their growing capacity; 'Eckehard', 'Gerburg' and 'Graf Wilhelm' are vigorously growing varieties.

- Fruit quality

One of the best varieties is 'Graf Wilhelm', a late winter variety with good appearance, but a poor yielder meant for lovers of pear fruits or enthusiasts only. 'Uta', 'Thimo', 'Elektra', 'Armida' and 'Isolda' have an excellent taste; all other varieties are good and better than 'Conference'.

- Yield capacity

The yield capacity is from high ('Gräfin Gepa', 'Graf Dietrich', 'Gerburg', 'Thimo', 'Elektra', 'Uta') to very high ('Eckehard', 'Hortensia'). 'Gerburg', David' and 'Armida' yielded only average.

- Ripening time and storability

'Hermann' ripens very early followed by 'Isolda'. They ripen before 'Clapps'. Within the group of autumn varieties, 'Graf Dietrich' 'Elektra' and 'Thimo' can store to November/December. 'Manon' ripens in September. The winter varieties in cold storage last until February/March ('Eckehard', 'Graf Wilhelm', 'Uta',) and March/April ('David'). The picking time and the storability vary greatly under different climatic conditions. We tested the storability only in cold storage, not under CA conditions. It may be possible to store 'Uta' and 'David' under CA conditions up to April (5–6 month). 'Eckehard', 'Gerburg' and 'Uta' obtain the best eating quality in December to February. Some of the improved varieties are given in Fig. 1.

- Compatibility

All varieties grow on seedling rootstocks and on quince with interstem 'Beurre Hardy'. The direct compatibility with quince is not yet tested for all varieties. 'Uta' and 'Isolda' are incompatible with quince in direct grafting.

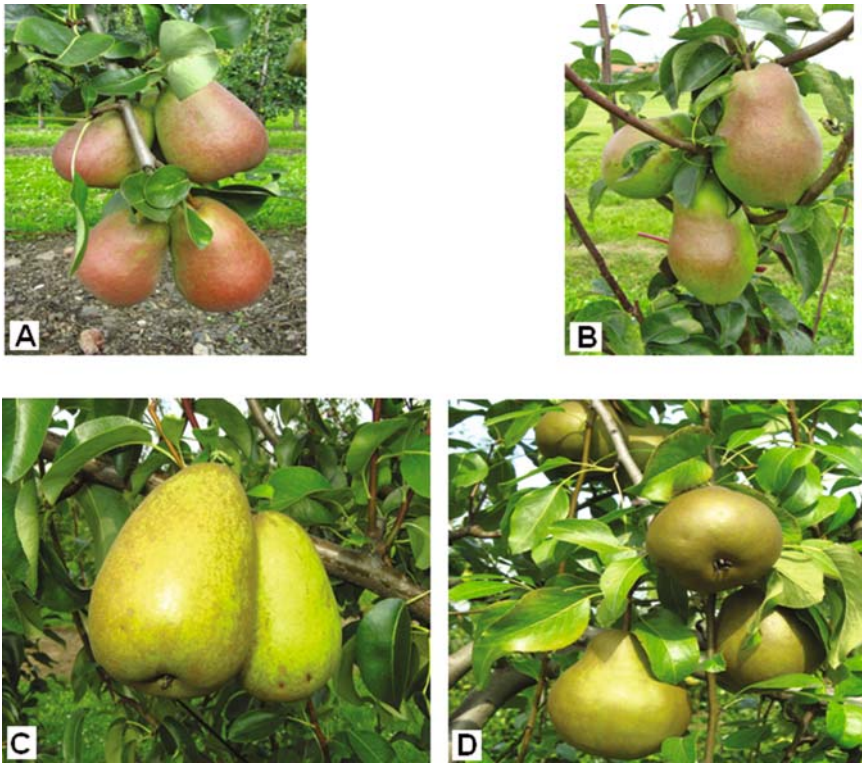

Fig. 1 A few improved varieties of Pears: Eckehard (**A**); Elektra (**B**); GrafWilhelm (**C**) and Uta (**D**) (*See* Color Insert)

- Resistance

We found no scab (*V. pirina*) and no *Pseudomonas* infection (out of 'Manon') in any of the named varieties. Varieties with pear decline and scab infection were eliminated. 'Isolda', 'Manon' and 'Uta' are only slightly infected by fire blight after artificial inoculation; all other varieties are susceptible to fire blight. The field test took place under a limited plant protection program, not without such measurements.

- Winter frost damage

After temperatures of −28°C, only a small damage occurs in wood or on buds of the new Pillnitz varieties 'Armida', 'David', 'Uta' and 'Eckehard', and in the Nashi pear from Japan, 'Shinseiki', 'Conference' and the new variety 'Concorde' from UK. The damage gives an indication of the reaction of varieties under extreme temperature stress conditions, but it is not a final characterisation of the frost resistance of the varieties (Fischer and Mildenberger 1998, 2000, 2004).

4.4.3 Short Description of some Recommended New German Pear varieties

Armida™ *
Origin: 'Jules Guyot' × 'Comice'
Tree: very dwarfing growth with good ramification, flat crown
Maturity: autumn, after 'Bartlett'
Quality of fruits: good taste, but on unsuitable soils, formation of stone cells is noted
Fruit size: large, oblong, slim, under-colour green, over-colour yellow, 170 g
Yield: middle to high, early bearing, mostly regular, not biennial
Resistance: good resistance against spring frosts, tolerant to scab or mildew infection, susceptible to fire blight
Pollination: diploid, good pollinators include 'Bartlett', 'Conference', 'Clapps Favourite', 'Hortensia'

David®
Origin: 'Jules Guyot' × 'Comice'
Tree: dwarfing growth with good ramification, flat pyramidal crown
Maturity: late autumn, with 'Alexander Lucas', storable to March
Quality of fruits: good after storage, very good transportability after picking
Fruit size: large, skin green, after storage it turns yellow-green, 180 g
Yield: middle, early bearing, regular
Resistance: no scab or mildew infection, susceptible to fire blight
Pollination: diploid, good pollinators include 'Bartlett', 'Anjou', 'Uta', 'Clapps Favourite', 'Paris'; for a good fruit set needs high temperature in the time of pollination; bad fruit set with 'Hortensia' and 'Conference'

Eckehard™ *
Origin: 'Nordhäuser Winterforelle' × 'Clapps Favourite'
Tree: vigorous growth, flat pyramidal crown
Maturity: winter, a few days before 'Alexander Lucas', storable until February/March
Quality of fruits: good to excellent, flesh in some years a little coarse with some grit cells
Fruit size: large to medium, skin under-colour green, over-colour to 50% vermilion to brown-red, 250 g
Yield: very high, early bearing, without fruit-thinning tendency to a less alternate bearing
Resistance: no scab or mildew infection, susceptible to fire blight
Pollination: diploid, pollinators include 'Bartlett', 'Clapps Favourite', 'Conference', 'Tongern', 'Anjou', 'Uta', 'Paris'

Gerburg™ *
Origin: 'Clapps Favourite' × 'Nordhäuser Winterforelle'

Tree: vigorous growth, pyramidal crown, needs to form up the branches for earlier beginning of cropping and higher yield
Maturity: late autumn, a few days after 'Conference', storable to December/January
Quality of fruits: good, aromatic, juicy
Fruit size: large, skin under-colour green –-yellow, over-colour to 60% red, attractive, 300 g
Yield: middle to low, late beginning of cropping
Resistance: no scab or mildew infection, susceptible to fire blight
Pollination: diploid, pollinators include 'Bartlett', 'Clapps Favourite', 'Uta', 'Tongern', 'Conference', 'Anjou', 'Paris', 'Hortensia'

Graf Dietrich® *
Origin: 'Clapps Favourite' × 'Nordhäuser Winterforelle'
Tree: vigorous growth, pyramidal crown, ramification loose, needs to form up the branches for earlier beginning of cropping
Maturity: autumn, like 'Conference', edible to December
Quality of fruits: very good, aromatic, juicy, for transportation needs early picking
Fruit size: medium to large, skin under-colour green, over colour yellow to brownish-yellow to vermilion, attractive, 250 g
Yield: middle to high, early bearing, better on quince than on seedling
Resistance: no scab or mildew infection, susceptible to fire blight
Pollination: diploid, pollinators include 'Bartlett', 'Clapps Favourite', 'Uta', 'Tongern', 'Paris', 'Hortensia'

Graf Wilhelm™ *
Origin: 'Comice' × 'Nordhäuser Winterforelle'
Tree: medium to vigorous growth, pyramidal crown
Maturity: late autumn, a few days before 'Alexander Lucas', storable to February/March
Quality of fruits: excellent, aromatic, juicy, for transportation needs early picking
Fruit size: large, skin under-colour green-yellow, over-colour yellow-brownish, attractive, 250 g
Yield: middle to low, form up the shoots can be helpful for a better cropping
Resistance: no scab or mildew infection, susceptible to fire blight
Pollination: diploid, pollinators include 'Bartlett', 'Uta', 'Conference', 'Paris', 'Hortensia'

Gräfin Gepa™ *
Origin: 'Nordhäuser Winterforelle' × 'Baierschmidt'
Tree: medium growth, pyramidal crown
Maturity: early autumn, a few days before 'Bartlett'

Quality of fruits: very good, aromatic, juicy, for transportation needs early picking
Fruit size: medium to large, skin under-colour green, over-colour to 90% red to vermilion, attractive, 220 g
Yield: middle to high, early bearing
Resistance: no scab or mildew infection, susceptible to fire blight, wood a little susceptible to winter frost
Pollination: diploid, pollinators include 'Bartlett', 'Clapps Favourite', 'Conference', 'Anjou', 'Uta'

Hermann® *
Origin: 'Jules Guyot' × 'Bunte Juli'
Tree: medium growth, flat pyramidal crown
Maturity: summer, a few days after 'Bunte Juli', no storable
Quality of fruits: good, aromatic
Fruit size: medium to small, skin under-colour green, over-colour to 20% brown-red, 150 g
Yield: middle to high, early bearing
Resistance: no scab or mildew infection, susceptible to fire blight
Pollination: diploid, pollinators include 'Bartlett', 'Clapps Favourite', 'Conference', 'Tongern', 'Anjou', 'Hortensia'—incompatible with 'Uta'

Hortensia®
Origin: 'Nordhäuser Winterforelle' × 'Clapps Favourite'
Tree: medium to vigorous growth with good ramification, flat pyramidal crown
Maturity: late autumn, a few days before 'Conference'
Quality of fruits: good
Fruit size: large, skin under-colour green-yellow, over-colour to 75% red to brown red, attractive, 220 g
Yield: very high, early bearing, regular
Resistance: no scab or mildew infection, susceptible to fire blight
Pollination: diploid, good pollinators include 'Paris', 'Clapps Favourite', 'Bartlett', 'Anjou', 'Conference'

Isolda
Origin: 'Jules Guyot' × 'Bunte Juli'
Tree: medium growth with good loose branch structure, flat pyramidal crown
Maturity: very early, a few days after 'Bunte Juli'
Quality of fruits: good to excellent
Fruit size: large to medium, skin under-colour yellow to green-yellow, over-colour yellow, to 20% vermilion (not in all years), 180 g

Yield: high, early bearing, regular
Resistance: no scab or mildew infection, susceptible to fire blight
Rootstocks: not directly compatible with quince
Pollination: diploid, good pollinators include 'Anjou', 'Clapps Favourite', 'Bartlett', 'Conference', 'Tongern', 'Paris'

Manon™ *
Origin: 'Beurre Bosc' open pollinated
Tree: medium to dwarf growth with good loose branch structure, flat pyramidal crown
Maturity: middle of September
Quality of fruits: good to excellent
Fruit size: large, skin under-colour yellow to green-yellow, over-colour gold-bronze a little russeting, 250–300 g
Yield: medium, regular
Resistance: no scab or mildew infection, susceptible to *Pseudomonas*, susceptibility not too high to fire blight
Pollination: diploid, good pollinators not yet tested

Thimo®*
Origin: 'Nordhäuser Winterforelle' × 'Madame Verte'
Tree: vigorous growth, ramification loose, needs to form up the branches for earlier beginning of cropping
Maturity: late autumn, like 'Conferece', storable to December/January
Quality of fruits: good, aromatic, juicy, a little coarse
Fruit size: medium to large, skin under-colour green-yellow, over-colour to 50% vermilion, attractive, 190 g
Yield: high, starts early, alternate bearing possible
Resistance: no scab or mildew infection, susceptible to fire blight
Pollination: diploid, good pollinators include 'Bartlett', 'Anjou', 'Uta', 'Clapps Favourite', 'Conference', 'Paris', 'Hortensia'

Uta®
Origin: 'Madame Verté' × 'Beurre Bosc'
Tree: dwarfing growth with good loose ramification, pyramidal flat crown
Maturity: winter, like 'Alexander Lucas', storable until February/March
Quality of fruits: excellent, very good transportability
Fruit size: large, skin under-colour green, 100% gold-bronze russet, very attractive, 280 g
Yield: very high, early beginning, regular
Resistance: no scab or mildew infection, only low susceptibility to fire blight, after full crop somewhat susceptible to winter frost
Rootstocks: not directly compatible with quince rootstock

Pollination: diploid, good pollinators include 'Clapps Favourite', 'Bartlett', 'Conference', 'Tongern', 'Paris'—incompatible with 'Anjou', 'Armida' and 'Hermann'

*These varieties are registered under the label 'SAXONIA'^{TM}-variety.

5 New Pear Varieties International

It is very difficult to introduce new varieties into the market. The main varieties worldwide are 'Bartlett' ('Williams'), 'Conference', 'Beurre Bosc', 'Abate Fetel', 'Anjou' (USA) and 'Comice'. The trade is not very flexible and does not accept many changes. Introduction of new varieties is more in local markets rather than in global markets. Nevertheless, new varieties are needed for more variation in supply, better resistance, better storability and longer shelf life. Well-adapted varieties are very important to the different climatic and soil conditions. Every nationally or regionally organised breeding program has its own legitimacy on this basis. Cosmopolitan pear varieties are very few and are the result of cross-combination breeding. New varieties introduced into the market are listed in Table 5.

- From *Italy* come dwarf growing, more or less tolerant to fire blight and pear psylla varieties: 'Tosca', 'Turandot', 'Norma', 'Carmen'.
- The *Canadian* breeding program is focussed to breed fire blight resistant varieties. Ready for tests are 'Harrow Delicious', 'Harrow Red', 'Harobig', 'Harrow Gold', 'Harrow Crisp'.
- Different institutions in *USA* bred especially for better hot climate adapted varieties: 'Elliot', 'Gourmet', 'Potomac', 'Summercrisp', 'Blacke's Pride', 'Rubia', 'Red Satin', 'Red Jewel', 'Red Spot', 'Red Silk'.
- The *Russian* breeding aimed frost-resistant and scab-tolerant varieties: 'Krasavitsa Chernenko', 'Bronzovaja', 'Svetljanca', 'Yanvarskaja', 'Smugljanca'.
- Further, intensive breeding programs at present take place in Estonia, Latvia, especially varities for better adaption to climatic conditions. Latvia, Estonia print in italics.
- *Czech Republic* are bred 'Bohemica', 'Erika', 'Dicolor', 'Delta', 'Decora', 'Dita', 'Jana', 'Omega', 'Barbara'.
- From *France* come 'Angelys', 'Delmire', 'Delwini', 'Delsavor', 'Delbuena', 'Bronstar', 'Beauroutard'.
- From a co-operative breeding program of *Switzerland* and *Great Britain* derived the more or less resistant to scab and mildew varieties 'Valerac' and 'Champirac'.
- In *Romania* were bred the disease-tolerant varieties 'Haydea', 'Monica', 'Euras', 'Getica', 'Daciana', 'Carpica' and 'Ina Estivale'.

Furthermore on pear breeding are working in Switzerland, Sweden, Moldova, Poland, Belgium, Australia, India, New Zealand, South Africa,

Table 5 New pear varieties

Variety	Origin	Growing capacity	Susceptibility	Flowering	Yield	Fruit	Comment
Angelýs®	France, 1963, INRA 'Winterdechant' × 'Comice'	Semi vigorous	Moderate to fire blight	Medium	High to medium	Sweet, juicy, fine aromatic, medium crisp	Eating time 11 to 3, needs optimal conditions for growing
Benita™ (Rafzas®)	Switzerland, 1996, 'General Leclerc' × 'Hosui'	Vigorous	Scab not yet, fire blight	Medium	Medium to high	Juicy, no much aroma, sweet	Eating time 9 to 11, for home gardener
Concorde®	Great Britain, 1993, 'Comice' × 'Conference'	Semi-vigorous, upright	Moderate scab, fire blight	Medium to late	High, regular, early beginnig	Good aroma and flavour, fine, crisp	Eating time 11 to 3, for direct selling
Condo	Netherlands, 1965, CPRO, Wageningen, 'Conference' × 'Comice'	Semi-vigorous, upright	Chlorose, moderate scab and spring frost, fire blight	Medium, it is a good pollinator	Medium, regular, early beginning	Juicy, sweet, aromatic, short shelf life	Eating time 10 to 2, for direct selling
Dessertnaja	Crimea, 1970, 'Boscs' × 'Olivier de Serres'	Semi-vigorous, good for slender spindle	Fire blight	Medium	Medium, regular, needs fruit thinning	Juicy, crisp, good aroma and taste	Eating time 8 to 9, for direct selling and home gardener
Highland®	USA, 1944, 'Bartlett' × 'Comice'	Semi-vigorous	Moderate scab, fire blight	Late	High	Only under optimal conditions juicy and aromatic	Needs very good conditions for growing, eating time 9 to 11

Table 5 (continued)

Variety	Origin	Growing capacity	Susceptibility	Flowering	Yield	Fruit	Comment
Harrow Sweet®	Canada, 2000, 'Bartlett' × ('Old Home' × 'Early Sweet')	Semi-vigorous, good ramification	Resistant to fire blight and pear psylla	Medium, it is a good pollinator	Medium to high, needs fruit thinning	Sweet, fine, aromatic	Fire blight resistant, difficult in growing, eating time 10 to 12
Verdi™ (Sweet Blush®)	Netherlands, 1997, 'Gute Luise' × 'Comice'	Vigorous	Fire blight, spring frost	Medium	Medium to high, needs fruit thinning	Very juicy, aromatic, fine	Juicy, eating time 9 to 11, incompatible with quince rootstocks

Chile, Argentina (*P. communis*) and Japan, China and Korea (*P. pyrifolia*) (Schuricht 1995; Hunter and Layne 1999; Paprstein and Bouma 2000; Andreies 2002; Bell and Puterka 2004; Fischer and Weber 2005).

6 Pear Rootstock Breeding

6.1 Breeding Aims and Methods

The main breeding aims for pear rootstocks are

- dwarfing
- free standing of trees
- precocity and productivity of varieties
- high yield efficiency
- positive influence to fruit quality and size
- efficient propagation ability
- compatibility
- cold hardiness
- tolerance to iron and calcium chlorosis
- resistance to fire blight
- resistance to pear decline
- no spins on the layers

In rootstock breeding dominates the European pear, *P. communis*, and quince, *Cydonia oblonga*. For Asian pears, other species are used: *P. pyrifolia, P. pashia, P. calleriana, P. ussuriensis, P. betulifolia*. All species without *P. communis* and *P. pyrifolia* used as rootstocks have problems with incompatibility (Jones and Aldwinckle 1990; Zwet et al. 1988).

Most of the breeding programs are based on combination breeding methods. They use cross-combinations between

- *P. communis* × *P. communis*
- *P. communis* × wild species
- *Cydonia* × *Cydonia*

The selection steps of vegetative propagated rootstocks are the following:

- seedling plants (morphology, phenology, rooting, resistances)
- mother plants in stoolbed (propagation ability, resistances, morphology)
- test for alternative propagation methods (in vitro, green cuttings, seeds)
- nursery tests in combination with varieties (compatibility, growing capacity, resistances)
- field tests in combination with varieties (yield, precocity, fruit quality, compatibility, dwarfing, tolerance to calcium and iron chlorosis, steadiness, healthiness)

Only few efforts were known in mutation breeding or clonal selection. Some quince selections, for instance, were found by using these methods. Seedling rootstocks can be selected by evaluation of populations after open pollination. 'Kirchensaller Most' and 'Fieudiere' are samples of successful election of donor varieties for seedling rootstocks (Wertheim 2000; Weber 2001; Webster 2003; Brown 2003).

New techniques, which can reduce the time scale for breeding a new rootstock in the future, will be very important. Currently, the marker-assisted selection techniques would appear to offer the most promise, although it will inevitably take some years to develop. If successful, and markers for useful characteristics, such as dwarfing, induction of scion cropping, pest and disease resistance and graft compatibility, can be developed, the techniques could significantly reduce the time and costs of rootstock breeding. The field evaluation would still be needed in the final phase of selection.

Genetic engineering can help to find more resistant rootstocks (fire blight) and new agronomic possibilities in growing herbicide resistance of the rootstocks. Some years of intensive research are still needed for its practical use (Lebedev et al. 2002; Dondini et al. 2002; Tartarini and Sansavini 2003).

Early selection methods could help to shorten the long time for selection especially in resistance tests for biotic and abiotic damages and in stoolbed performance.

6.2 New Rootstocks International

For intensive plantations, dwarfing vegetatively propagated rootstocks (clonal rootstocks) is needed. Internationally, it is much more difficult than in apple rootstock selection. In USA, the OH × F-rootstocks were selected (crossings between the fire blight resistant varieties 'Old Home' × 'Farmingdale'). There is not enough dwarfing, but it is very important to have resistance against fire blight, pear decline and calcium chlorosis.

The 'Perry' pears from France (RV 139) possesses medium growing capacity; they are well suited for trees in landscape and not in intensive plantations. The 'Retuziere' rootstock series derived from the variety 'Old Home' (OH 11, 20, 33) grows like quince A and is free standing. The BP rootstocks from South Africa and the Fox rootstocks from Italy grow vigorously. 'Pyrodwarf' from Germany (Geisenheim) and some OH rootstocks from France ('Pyriam' = OH 11) have a medium fire blight resistance and they grow moderately. They are compatible with most of the varieties and are easy to propagate, but the fruit size of varieties on these rootstocks is smaller.

Some institutions are involved in selection of quince rootstocks. Important are the Polish activities for more frost-resistant quince rootstocks (Quince 'Sydo'). All others focussed the work for better compatibility, more dwarfing, better resistance to pests, tolerance to chlorosis and better free standing of trees (Great Britain, France, Italy). Not all attributes could be realised in a single genotype. In comparison to apple rootstocks, pear needs much more breeding and field test work. Quince has not enough resistance against frost and fire blight and not all varieties are compatible with quince. All other rootstocks have deficits in dwarfing, free standing and disease resistance. Therefore, though new rootstocks have advantages in many characteristics, it is still a long way to achieve the 'ideal' rootstock (Grzyb 1987; Fischer 1996; Wertheim 2000; Jacob 2002; Webster 2003; Fischer and Weber 2005).

6.3 Rootstock Cross-Combination Breeding in Germany

The aims of the German pear rootstock breeding program had been to improve propagation dwarfing, resistance to biotic and abiotic damages, sufficient anchorage, positive influence on yield and fruit quality of the varieties, and free of suckers and burr knots. Results were received from long-term randomised trials and from field tests in farms under different production conditions. Approximately 6000 seedlings borne out of crosses between wild species and known pear varieties were grown. Finally, seven clones were selected (Table 6).

The new Pillnitz pear rootstocks are moderate to propagate in stoolbeds, but easy by green cuttings under mist (Table 7) and in vitro. They are more frost resistant against winter frost than quince rootstocks, and the growing capacity is intermediate between quince and seedling.

Pomological testing with 'Clapps Favourite' was done under a minimal pruning regime to evaluate the cropping potential. Compatibility with several

Table 6 New German pear rootstocks (Dresden-Pillnitz)

Rootstock (breeding no.)	Growing capacity	Propagation	Parents
Pi-BU 1 (IID 2-68)	Medium–strong	Easy	Clapps F. × *P. longipes*
Pi-BU 2 (523-15)	Medium–dwarf	Easy	Clapps F. × *P. longipes*
Pi-BU 3 (IID 7-109)	Dwarf	Medium	*P. longipes* open pollinated
Pi-BU 4 (A 26-86)	Medium–strong	Medium	*P. pyrifolia* open pollinated
Pi BU 5 (IID 11-120)	Very dwarf	Easy	*P. sinaica* × *P. pyrifolia*
Pi BU 6 (IID 20-68)	Medium–strong	Medium	*P. bretschneideri* × *P. sinaica*
Pi-BU 7 (IID 5–52)	Medium	Easy	*P. pyrifolia* open pollinated

Table 7 Rooting of green cuttings of *Pyrus* progenies under mist

Cross-combination	No. of Years	Clones	Rooted cuttings (%)	Root evaluation 0 (without) to 5 (+)
Clapps F. × *P. elaeagrifolia*	9	4	60.5	3.9
Bartlett × *P. elaeagrifolia*	6	4	49.5	4.2
J. Guyot × *P. sinaica*	7	4	43.7	2.7
P. aromatica× *P. sinaica*	14	2	86.1	3.6
P. sinaica × Nordhäuser	3	3	86.0	3.1
P. sinaica × *P. heterophylla*	5	3	73.5	2.4
P. nivalis × M. Verté	5	3	13.3	2.5
P. nivalis × *P. longipes*	35	2	85.2	3.1
P. longipes × *P. nivalis*	5	3	87.7	2.9
P. sinensis × J. Guyot	18	2	94.1	4.0
P. ussuriensis × *P. pyraster*	11	2	90.1	2.5

varieties was satisfactory. Promising clones were quickly multiplied using in vitro propagation. At present, new pear rootstocks undergoing field tests are Pi-BU 2 and Pi-BU 3 together with the new German rootstock from Geisenheim, 'Pyrodwarf'.

Most of the populations were tested and selected after artificial inoculation with aggressive strains of fire blight, *E. amylovora*. The results are listed in Table 8. Only two populations were found with moderate infected progenies: *P. canescens* × *P. serrulata* and *P. betulifolia* × *P. ussuriensis*. *P. canescens* and *P. serrulata* are described as susceptible to fire blight. Less susceptible progenies can also segregate from susceptible parents. Evidently, an accumulation of resistance genes is necessary for an expression of idiotypic resistance to fire blight. This indicates a polygenic resistance. Apparently, *P. betulifolia* and *P. ussuriensis* are carriers of blight-resistance genes and can be used in resistance breeding furthermore.

First results in testing the growing capacity of the new German rootstocks demonstrate that they are dwarf, but not dwarf enough for intensive plantations. The dwarfness of the new rootstocks was tested in nursery and field tests with different varieties (Fischer 1969, 1996, 2004). Results of nursery tests are compared in Fig. 2.

Table 8 Fire blight on populations and clones of Pyrus progenies

Population	Resistance evaluation: 1 = totally infected; 9 = resistant		
	1. Test	2. Test	3. Test
Clapps Favourite × *P. longipes*	–	1,0	1,1
Clapps Favourite × *P. pashia*	–	–	1,7
Comice × *P. pashia*	1,2	1,6	–
P. betulifolia × *P. communis* var. *caucasica*	2,9	1,4	–
P. pyrifolia × *P. communis* var. *caucasica*	1,0	1,2	1,6
P. pyrifolia × *P. longipes*	1,3	1,2	–
P. longipes × *P. nivalis*	1,0	1,0	–
P. nivalis × *P. longipes*	1,0	1,0	–
P. nivalis × *P. calleryana tomentella*	–	1,0	1,0
P. aromatica open pollinated	1,8	2,1	1,6
P. sinaica × Nordhäuser	1,0	1,5	3,4
P. sinaica × J. Guyot	1,9	1,5	–
P. aromatica × *P. sinaica*	2,6	1,3	1,7
P. regelii × Kieffer	1,1	1,2	–
P. communis × *P. sinaica*	1,0	1,0	–
P. X bretschneideri × *P. sinaica*	1,0	–	1,6
P. X bretschneideri × *P. salicifolia*	1,2	–	–
P. X bretschneideri × Kieffer	1,0	–	1,6
P. X bretschneideri × *P. pyraster*	1,2	1,3	1,4
P. X canescens × *P. serrulata*	8,3	–	4,3
P. X canescens × *P. betulifolia*	1,7	–	1,6
Verté × *P. betulifolia*	–	1,3	–
P. communis × *P. betulifolia*	–	1,3	1,3
P. betulifolia × *P. ussuriensis*	2,6	6,0	–
P. communis × *P. ussuriensis*	–	1,0	1,0
P. ussuriensis × *P. pyraster*	1,3	1,0	3,4
P. pyraster open pollinated	1,0	1,0	1,4
P. pyrifolia open pollinated	1,0	1,0	–
Quince BA 29	1,5	–	–
Quince A	1,0	1,0	–
OHF 333	5,7	–	–
Kirchensaller Most, seedlings	1,0	–	–
Pyrodwarf	4,2	–	–
Pi-BU 2	2,0	–	–
Pi-BU 3	1,7	–	–

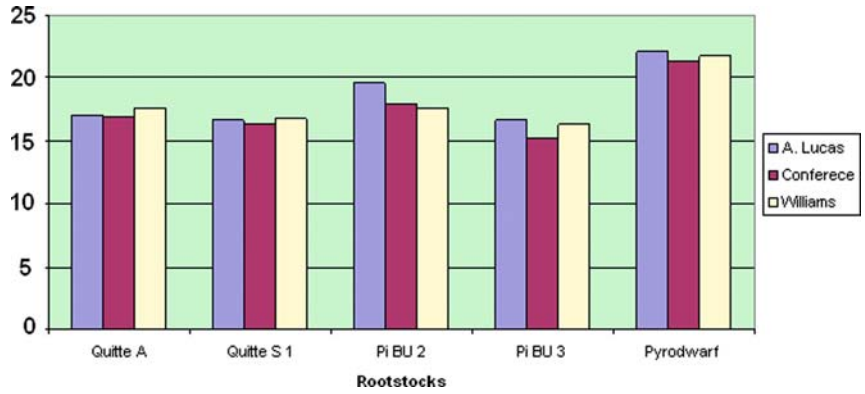

Fig. 2 Trunk diameter of 2-year-old maiden trees of three varieties on five rootstocks (*See* Color Insert)

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Plum Breeding

Walter Hartmann and Michael Neumüller

1 Introduction

1.1 Origin of European Plum

A wild type of *Prunus domestica* (European plum) and especially the typical form of this species, the prune, is unknown. Crane and Lawrence (1934) suggest that the hexaploid *P. domestica* is a hybrid of *P. cerasifera* (cherry plum, diploid) and *P. spinosa* (sloe, autopolyploid tetraploid). The blue fruit colour is coming from the sloe; the other colours are originating from the cherry plum. Rybin (1936) found spontaneous intraspecific hybrids in the Caucasian region. One of this natural hybrids had $2n = 48$ chromosomes and was morphologically indistinguishable from the common plum. He crossed *P. spinosa* and *P. cerasifera*, and obtained seedlings regarded as re-synthesised *P. domestica*. Most of these plants were sterile and had no or only some fruits. However, one hybrid was highly fertile; this supported Crane's assumption of the origin of *P. domestica*. A similar experiment was made by Endlich and Murawski (1962). The hybrid nature of *P. domestica* is nowadays widely accepted, and it is assumed that the species originated in the Caucasian region because *P. cerasifera* and *P. domestica* are native there.

The natural hybrid found by Bajashvili (1991) in the Caucasus region of Georgia confirmed once more the hypothesis of the origin. However, he could not exclude another way of origin; for example, the mentioned genotype could

W. Hartmann
University of Hohenheim1, Institute of Special Crop Cultivation and Crop Physiology, Emil-Wolff-Straße 25, 70599 Stuttgart, Germany
e-mail: walthart@uni-hohenheim.de

M. Neumüller
Technishe Universität München, Fachgebiet Obstban, Dürnast 2, 85354 Freising, Germany
e-mail: neumueller@obstzentrum.de

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have been an autopolyploid seedling of a cultivated form of *P. cerasifera* or of *P. spinosa*. Salesses' study (1973) shows that there are two non-homologous genomes present in *P. spinosa*. This is an evidence that this species is allopolyploid. In cytological studies, he stated that there is a homology between the genomes of *P. spinosa* and *P. cerasifera*. In an analysis of a restriction map of the ribosomal genes, Reynders and Salesses (1990) concluded that *P. spinosa* is most probably of allotetraploid origin but the study does not permit the establishment of a definitive map.

The origin of *P. domestica* dates back millenniums of years. As it is assumed that the Caucasus is the origin of *P. domestica*, its wild ancestor should be found there. Surprisingly and inexplicably, no wild types of *P. domestica* were found in this area neither by Rybin (1936) nor by Bajashvili (1991).

In field observations, Bajashvili (1991) found not only hybrids between myrobalanes and sloes but also polyploid myrobalanes and sloes with more than four chromosome sets. Some of these sloes are distinguishable from the common tetraploid ones by their larger fruits, their sweet taste and the lack of astringency. The size of leaves and buds are close to that of cultivated plums. Others show some features of myrobalan. He presumes that these sloe genotypes originated not only by polyploidisation but also by spontaneous hybridisation between sloe and myrobalan.

All the research work done so far cannot give a clear answer to the origin of *P. domestica*, but it is accepted that *P. spinosa* and *P. cerasifera* are involved in the genesis of European plum. More information may be obtained using modern tools of molecular biology.

1.2 History of European Plum

The plum has been the first species among all fruits to attract human interest (Faust and Surányi 1999). The spontaneous occurrence of *P. domestica* has taken place some time before the Neolithic Age. Its oldest remains are stones of the subspecies insititia. They were found both in the Ukraine dating back 6 000 years (Erményi 1975/77) and in the South of Germany near Ulm (4060–3956 BC). According to Knörzer (1974), the damson seeds found in the Neolithic Age in Germany must come from cultivated plums because at this time no wild damsons were present there.

The European plum was first mentioned in the 7th century BC in Archilochus's 'Pollux'. Theophrast (4th cent. BC) mentioned the name 'Prumnon' for the first time. With this name, the species came to the Romans and the name changed to *Prunum*. Plinius (1st cent.) reported about many cultivars with fruits in yellow, red, violet, black, white or bright colours. Also the grafting of plums on sloe has already been known at this time.

The Romans brought the plum in the region north of the Alp Mountains. A 'taverna' was found there at the north of Lake Balaton in Hungary with a wall paint from 1st century illustrating a woman's head surrounded by reddish purple plums. According to Ramming and Cociu (1991) and Faust and Surányi (1999), large plum orchards were established on the banks of the rivers Drava and Sava between the second and third century. Since then, Bosnia is leading in plum production in Europe, especially for the cultivar 'De Bosnia', a synonym of the variety 'Pozegaca', which is today the most important prune variety in Europe. This variety was introduced in Germany in the 17th century and was called 'Zwetsche' or 'German Prune' in contrary to the existing plums. The same variety was mentioned in a Latin language document in Hungary in the year 1552: 'Una libra pruni Beszterci [. . .]' (Tòth and Surányi 1980).

The origin of 'Prune d'Agen' also dates back to this time. Benedictine monks planted the 'Date Plum' in their garden in the vicinity of Bordeaux in France (Hedrick 1911), first called 'Prune d'Ente' and later 'Prune d'Agen'. In the meantime, a lot of hybrids have been introduced.

The origin of the reine-claude-group is unknown. Plums, very similar to reine-claudes, were found in the trans-Caucasian region by Koch (1876), and therefore, he assumed that this group originated in this region. The name 'reineclaude' goes back to a plum named after Queen Claudine in 1525, who was married with the French King Francis I (1494–1547). Since then, it has been cultivated as 'Reine Claude Verte' in France.

There are different opinions about the systematic position of mirabelles. Taking into accout the feature of their fruits and the tree, it cannot be a hybrid of *P. cerasifera*. Nevertheless, many people are mixing up the fruits of the two species. Also the word 'Mirabelle' is not coming from myrobalan; it is assumed that it is derived from the French word 'mirable' that means 'wonderful'. Mirabelles are originating probably in Asia Minor or Armenia and were brought to the Mediterranean area by the Romans. Since a long time, the largest area of cultivation is Lorraine in France. Mirabelles were introduced there by the provincial monarch René (1409–1480).

A detailed and profound description of the history of plums was made by Faust and Surányi in 1999. In France, many plum cultivars were known in the 17th century. In 1628, Le Lectier described 55 varieties. During the 19th century, many cultivars were introduced. Liegel (1861) listed more than 290 varieties, but all of them resulted from open pollination and natural or man-made selection. The systematic breeding was started by Th. Rivers (England) in 1843 and at the end of the 19th century by Mitschurin (Russia). Eighty ancient varieties are described by Caillavet (1991). More than 1000 European plum cultivars are mentioned in the literature (Hedrick 1911), but the number of those commercially used is limited. An account of the plums of England was given by Taylor (1949). A description of plum varieties of commercial interest was made by Basso and Faccioli (1978), Surányi and Erdös (1998) and Hartmann (2003).

1.3 Origin and History of Japanese Plum

The wild form of *P. salicina* is unknown as well. It may originate from Yangtze River Basin in China (Yoshida 1987). There is a cultivar named 'Zhui Li' dating back more than 2 000 years. Very early, *P. salicina* was introduced to Japan. Stones were found dating back to about 200 BC, and the cultivation of plums was mentioned around 500 AD (Yoshida 1987). *P. salicina* was introduced to Japan either over the Korean peninsula or by a Chinese monk, who brought plum trees as a gift to the Emperor (Matsumado 1977).

P. salicina was imported to California in North America by Hough in 1870. The first fruits of these trees were produced by J. Kelsey. A wide distribution of Kelsey's plum trees was made by W. P. Hammon & Co in 1884. He named the fruit 'Kelsey'.

The famous plum breeder Luther Burbank started his breeding in 1875 using all plum species available. He produced hundreds of thousands of seedlings and selected a lot of valuable varieties. Howard (1945) lists more than 100 plum cultivars introduced by Burbank. Some of them were imported, but most of them originated from his breeding work (open pollination or controlled hybridisation). In 1885, Burbank imported a cultivar with intensively red fruit flesh, known as 'Blood plum of Satsuma'. The most famous cultivar of the century introduced by Burbank was 'Santa Rosa', a complex hybrid descending from *P. salicina, P. simonii* and *P. americana* with predominating *P. salicina* character. Because of the reddish flesh, one of the ancestors is supposed to be 'Satsuma'. Burbank itself regarded the varieties 'Santa Rosa', 'Formosa', 'Beauty' and 'Wickson' as the best ones. His work was continued by several breeders. Okie and Weinberger (1996) list the breeders involved in the improvement of Japanese plum cultivars.

All modern Japanese plum cultivars are going back to some genotypes originated by hybridisation between *P. salicina*, *P. simonii* and native North American species, especially *P. americana, P. nigra, P. angustifolia, P. hortulana* and *P. munsoniana*. There is very little diversity among the Japanese plum cultivars because of inbreeding of existing cultivars. The top 10 cultivars of California with exception of 'French Prune' (an European plum cultivar) and 'Simka' trace back to five parents all released by Luther Burbank (Okie and Weinberger 1996).

Nowadays, none of the native North American species is commercially grown any more, and only a few of the improved selections are still available.

1.4 Fruits of Plums and Prunes and Their Use

Fruits of plums and prunes are used freshly, dried or canned. Fruits of *P. salicina* are nearly exclusively used for fresh consumption, and fruits of *P. domestica* additionally for processing. Plums are used to make jam, juice,

liquor and brandy, for baking and for confection. Concentrated juices of plums and prunes are also used for medicinal purpose as laxative. The fruits are of high benefit for human health. Depending on the cultivar, fresh plums have a sugar content of 7–23% with sucrose as the most important sugar followed by glucose, fructose and sorbitol. The content of sucrose depends on the cultivar and varies between 8 and 50% of dry matter. If fruits are used for brandy production, their content of sorbitol is very important because sorbitol is not fermentable. The sorbitol content depends not only on the cultivar but also on the yield of the tree and on climatic conditions. The higher the light intensity (sunshine) the more sorbitol is found. The content of sorbitol varies between 3 and 30% of dry matter. There is a positive correlation to the content of other sugars, but a negative to that of sucrose (Hartmann 1984).

Plum fruits have a high content of potassium and vitamin A and also vitamins which are not found in other fruits or only in traces such as vitamins B1 and B6, niacin and pantothenic acid. Because of their role as scavenger, phenolic compounds are an important factor for the health effect of plum fruits. The capacity to subdue free oxygen radicals can be quantified in *in vitro* experiments and shown as oxygen radical absorbance capacity (ORAC) value (Table 1).

The production of dried prunes is an old tradition in most European countries. It was neglected in the last decades, because Californian dried prunes are dominating the world market. In some countries, for example, in the Balkan region, drying is still an important economic factor. Predominantly, the variety 'German Prune' is used for this purpose. In the former Yugoslavia, up to 34 000 t plums per year were dried. In 1998, 911 t of dried prunes were exported amounting to 1 147 000 US$ (Sevarlic 2000). In Romania, on an average more than 9 500 t fresh fruits are used for drying in the years 1996–2005, which is 2.4% of the plum production worldwide (Botu and Cociu 2000).

Much more important than drying is the fermentation for brandy production. Plum brandy is famous in most European countries. Most of the fruits in Yugoslavia are used for the 'Slivovitz' production (up to 120 million litres per year) (Sevarlic 2000). In Romania, plums are traditionally used for distillery. In the last 30 years, the rate of harvested fruits used for brandy production

Table 1 ORAC value of some fruit species (Wang et al. 1996; McBride 1999)

Fruit species	ORAC units/100 g*
Apples (fresh)	218
Oranges (fresh)	750
Cherries (fresh)	670
Plums (fresh)	949
Raisins (dried)	2830
Prunes (dried)	5770

*Data expressed as micromoles of Trolox equivalents per 100 g fresh or dry matter

decreased from 80–85% to 55%. In the years 1991–1999, 216 000 t of plums have been processed in alcohol annually on an average (Botu and Cociu 2000). In Bulgaria, about 15% and in Hungary, 10–15% of the total production are used for brandy production.

1.5 Economic Importance of Plum Production

During the last decades, the world production of European and Japanese plums increased from 5 679 000 (average for the years 1969–1971) to 9 521 336 t in 2004, i.e., by 67%. In 1998, the plum production was ranked on the 10th place of the world fruit production with 6 590 000 t as well as in 2005 with 9 843 00 t. Considering only the temperate fruit crops, the plum takes the third position after the apple and the pear.

Most plums are produced in Asia with more than 5 265 800 t on the average of the years 2000–2004 (Table 2). Europe holds the second position ahead North America. The strong increase in plum production is due to the enlargement in China. On the average of the years 1975–1982, the Chinese production was only 460 000 t. It increased to 747 000 t on the average of the years 1986–1988 and to 4 427 000 t on the average of the years 2002–2004 (FAO Yearbook 2005). During the last 20 years, the production raised eightfold. However, these quantities given in the FAO Yearbook are in strong disagreement with Weisheng (2006). He estimates that the annual production (average of 2002–2004) has been 1 870 000 t.

In Asia, China is dominant in plum production followed by Iran and Japan. The plum-growing area in China was enlarged from 53 000 ha in 1985 to 240 000 ha in 1990 (Liu 2007) and to 1 448 000 ha in average of the years 2000–2005 (FAO Yearbook 2005).

In North America, about 90% of the market fruits are produced in California. The most important cultivar is 'French Prune'. In 1975, more than 70% of the commercial plum production accounted for this variety (Fogle 1978). Meanwhile, the production of Japanese plums rose up. The annual report for 2004 states that 36 000 ha bearing acres are for fresh plums (mainly Japanese) and 67 000 bearing acres for dried prunes, mainly with 'French Prune'. The value of

Table 2 Plum production (t) 2000–2004 (FAO Yearbook 2005)

Year	Europe	North America	Asia
2000	2 733 318	821 990	4 940 641
2001	2 793 452	593 680	5 073 494
2002	2 259 743	670 477	5 444 480
2003	3 467 946	739 836	5 458 369
2004	3 091 628	293 257	5 412 038
Ø	2 869 217	623 848	5 265 804

Table 3 Plum production (t) worldwide in important plum-producing countries (FAO Yearbook 2005)

Country	1988	1993	1998	2004
Argentina	58 800	53 000	78 228	127 413
Bosnia and Herzegovina	–	50 000	58 639	73 000
Chile	85 000	120 000	139 800	255 000
China	858 485	1 612 402	3 161 503	4 434 000
France	221 000	185 643	205 700	229 134
Germany	574 109	366 200	338 680	568 000
Iran	102 418	142 227	118 314	147 000
Italy	153 960	130 198	148 849	179 133
Japan	67 700	96 500	95 600	90 000
Korea, Republic of	31 990	20 372	39 006	77 438
Poland	97 688	98 847	107 132	110 000
Romania	534 200	703 700	404 370	475 767
Russian Federation*	954 000	149 800	105 000	178 000
Serbia and Montenegro†	765,353	519 000	481 000	561 000
South Africa	27 494	29 591	43 225	65 063
Spain	120 100	157 100	146 546	178 700
Turkey	175 000	200 000	200 000	200 000
Ukraine	–	224 000	72 900	130 000
USA	669 500	533 000	507 000	290 000
Uzbekistan	–	62 000	69 000	90 000
Total	5 496 797	5 453 580	6 520 492	8 458 648

*Data of 1988: USSR. †Data of 1988: Yugoslavia. - no data available.

production of dried prunes was 1 246 billion US-$ in average of the years 1995–2004. The decrease of production was caused by a decrease of the price for dried prunes of about 25% during the last 10 years (USDA-NASS 2005).

The leading producers in Europe are Germany, Serbia and Romania (Table 3). The production area and the yield per hectare of the most important plum-growing countries are given in Table 4. Mostly, yield in the orchards of fruit growers is much higher than the average yield, for example, in Germany between 15 and 30 t/ha.

Table 4 Production area and yield per hectare of the most important plum producers in the years 2000–2005 (FAO Yearbook 2005)

Country	Production area (ha)	Yield (t/ha)
China	1 448 988.67	3.0
UDSSR, former	152 031.67	3.5
Yugoslavia, former	173 083.33	3.2
Romania	94 402.17	5.5
Germany	63 333.33	7.5
USA	47 368.50	11.8

In Germany, plums are ranked in the second place in tree fruit production after apples. However, one has to distinguish between the total and the market production. Only about 10% of all plums are coming in the market. Nevertheless, also from the fruits put on the market, plum is the second important tree fruit species. A similar ratio of total and market production is found in many countries, because plum trees are growing mostly in house gardens or in the landscape where the fruits are not always harvested or used for brandy production. In Europe, there are considerable differences in the type of requested plum fruits: whereas in Middle and Eastern Europe, people prefer small- to middle-sized fruits with firm, tasty flesh and adequate content of organic acids, in other countries like Italy, the Netherlands, Great Britain, the Scandinavian countries and Poland, large-sized fruits are preferred. A special market for mirabelles and gage plums ('Green Gage') exists in France—there are about 3000 ha of each gage plums and mirabelles in production with an annual harvest of around 30 000 and 15 000 t, respectively (Chauvin et al. 1990; Audubert and Chambonniere 1995).

In Europe, about 90% of the plums produced are European plums, whereas in Asia 82% are Japanese plums (Suranyi and Erdös 1998). In North America, 53% of the produced plums are European, 38% Japanese and nearly 9% *P. americana, P. simonii, P. nigra* and *P. munsonia* (Surányi and Erdös 1998). Ramming and Cociu (1991) estimated the production of Japanese plums for different countries, for example, 40% for Italy, 55% for Spain, 85% for Australia and 99% for Pakistan. In China, 99% are Japanese plums as well (Weisheng 2006). For the individual country, the agricultural value addition of plum production is more informative than the production in metric tons. A comparison of the agricultural value added between different countries is hardly possible. For Germany, the agricultural value addition of plums is ranked on the second place in tree fruit production.

The amount of plums harvested fluctuates considerably from year to year. In many countries, plums are not cultivated at high production intensity, resulting in years with high yield followed by years with low yield. This alternate bearing depends on the cultivar as well and is often caused by bad weather condition or spring frost. For example, in Germany the lowest production in the period of 1995–2005 was in 1995 with 289,900 t and the highest in 2004 with 568 000 t (FAO Yearbook 2005). In Romania, the plum production was 130 800 t in 1951 and 738 000 t in 1959 (Cociu 1997a). The variation in the production of the most important plum-producing European countries in the years 2001–2004 is listed in Table 5. In the Eastern European countries, the range of variation is higher.

During the last centuries, there was a great change in the importance of plums and prunes in the European fruit production. At the end of the 15th century, more than 50% of all fruit trees in Germany were plums or prunes. In Romania, at the beginning of the 20th century nearly 90% of the fruit trees grown in orchards were plums and prunes, decreasing to 64% in 1959, 49% in 1980 and 37% in 1993 (Cociu 1997a).

Table 5 Variation in the plum production (t) in Europe in different years (FAO Yearbook 2005)

Western Europe	2001	2002	2003	2004
Austria	75 300	43 418	69 499	69 579
France	271 579	246 376	250 192	229 134
Germany	387 987	424 457	478 730	568 000
Italy	177 405	177 149	127 638	179 133
Spain	149 734	210 900	224 600	178 700
Total Western Europe	1 062 005	1 102 300	1 150 659	1 224 546
Eastern Europe	2001	2002	2003	2004
Bosnia and Herzegovina	26 750	58 000	88 308	73 000
Bulgaria	73 150	48 891	46 364	55,000
Hungary	89 824	49 316	45 430	45 000
Poland	131 888	102 892	109 563	110 000
Romania	557 200	220 638	909 648	475 767
Serbia and Montenegro	338 000	205 371	577 431	561 000
Total Eastern Europe	1 216 812	685 108	1 776 744	1 319 767

The most important plum production region in Europe is located in Eastern Europe, especially in the Balkan countries. Because of the problems with the Plum pox virus (PPV), which causes the Sharka disease, the production decreased considerably during the last decades, for example, in Bulgaria from the beginning of the 1970s to the 1980s by nearly 50% (Djouvinov and Vitanova 2000). The change in the political system caused further losses in the production. For a long time, the leading country in plum production worldwide was Yugoslavia with a number of 60 million trees in 1982 declining to 44.5 million in 1999 (Sevarlic 2000). Much more decreasing was the production in Poland from 20 million of trees in 1962 to 2 million in 1987. For this reason and because of the low prices for apple, the interest on plum production has been rising in Western Europe during the last 15 years.

In the last years, modern plum cultivation methods have been established in the Eastern European countries. For example, the number of plum trees in Poland was rising to 12.5 million trees in 1999 (Grzyb 2000).

2 Botany and Taxonomy

Plums are produced throughout the world. Hedrick (1911) commented that the range of fruit size and shape, flavour, aroma, texture and colour in plums is greater than that in any of our cultivated fruit species. The diversity of plums is also reflected in their names. There are plums, prunes, gage plums, egg plums and mirabelles, and the wild plums like cherry plums, bullaces, damsons and sloes. Three of the most impact species of plums are not known as wild species and were presumably selected and cultivated very early by men. Some of the

plum species originated in Asia, and others in Europe and in North America. Most of the cultivated plum cultivars belong to only two species—the European plum (*P. domestica*) with a hexaploid genome ($2n = 6\times = 48$) and the Japanese plum (*P. salicina*) with a diploid chromosome set ($2n = 2\times = 16$).

2.1 The European plum (*P. domestica* L.)

P. domestica is the most important plum in Europe, but it is also grown in other continents. This species is primarily cultivated in cooler regions and can be divided in several groups considering the fruit characters.

2.1.1 Plums

These fruits are round to oval in different sizes and colours. The flesh is juicy, soft and mostly clingstone. Usually, the fruits are ripening earlier than those of prunes, but there are also some exceptions of this rule.

2.1.2 Prunes

These fruits are oval to elongated, mostly smaller than plums and generally high in sugar content. They can, therefore, be well used for drying. The colour is mostly dark blue to purple, but there are also some cultivars with red, pink, yellow or bright colour.

The differentiation between these two groups is not easy, but in some cases necessary, for example, for import regulations and for different taxes in brandy production. After cooking, while the flesh of plums dissolves, the flesh of prunes remains firm. There are also differences in the pubescence of shoots and leaves. Those of prunes are never pubescent. The name plum is also considered as general term. A pertinent statement was made by Teskey and Shoemaker (1978): 'All prunes are plums but not all plums are prunes.'

Typical prunes are 'Prune d'Agen' and 'German Prune', which is the most spread prune in Europe, called 'Hauszwetsche' in Germany, 'Pozegača' in Yugoslavia, 'Beszterci' in Hungary, 'Casalinga' or 'Dro-Zwetsche' in Italy, 'Quetsche Commune' in France, 'Vinete romanesti' in Romania and 'Kustandilska' in Bulgaria, and in some countries known as 'Commun Plum'.

A description of plum varieties in Italy was made by Basso and Faccioli (1978). Surányi and Erdös (1998) give an overview of the plum varieties in Hungary and Ghena and Braniste (2003) in Romania. Plum and prune varieties of commercial interest in Germany are mentioned in Chapter 7, and a description of new varieties was given by Hartmann (1998, 2007) and Jacob (2007). Promising new plum cultivars are mentioned by Hodun et al. (1998) for Poland and by Kemp and Wustenberg (1998) for the Netherlands.

The related gage plums or reineclaudes and mirabelles are of lower economic importance. Botanically, the mirabelles have often been classified to *P. insititia*. Today they are regarded as a subspecies of *P. domestica*, but this is still under controversy.

2.1.3 Gage Plums

They are also called 'reineclaudes' with small to median round fruits in different colours from green ('Green Gage') to yellow ('Oullins') and purple ('Graf Althans'). The flesh is juicy, sweet, with aroma, very tasty and of high quality. The fruits are used mostly for fresh consumption.

2.1.4 Mirabelles

Mirabelles have small round fruits (8–12 g) and 22–28 mm in diameter, mostly yellow coloured, often with red spots, but there are also green and more purple-coloured varieties. The fruit is freestone, juicy, very sweet (18–20% Brix), full of aroma and of high quality. They are used especially for canning and brandy industry, but in the last time more and more for fresh consumption, too. Most famous are 'Mirabelle de Nancy' for the fresh market and 'Mirabelle de Metz' for brandy production.

2.1.5 Primitive Forms and Autochthonus Biotypes

In many countries, primitive, mostly local forms of *P. domestica* have been found. They are grown on their own roots and are propagated by suckers. In former times, they were cultivated, but nowadays they are only found growing in not cultivated hedges or on the skirts of forests. In Middle Europe, Spillinge are well known. They were described by Tabernaemontanus (1588) for the first time. Different autochthonous biotypes were found by Werneck (1958) in Austria. The resistance and quality characteristics of local and old plum cultivars and primitive landraces were described by Paunovic (1988). In Hungary, local plum genotypes were used for breeding purposes (Surányi 1998). In Bulgaria, local varieties were studied by Ivanova et al. (2002) and in Romania by Botu et al. (2002).

2.2 The Asian Plums

2.2.1 Japanese Plum (*Prunus salicina* Lindl)

Originally, the term 'Japanese plum' was used for varieties of *P. salicina* exclusively, but nowadays it includes all the fresh market plums developed by the hybridisation of various diploid species with *P. salicina* as well (Okie and

Weinberger 1996). The species is primarily cultivated in warmer regions because of its lower chilling requirements and its sensitivity to winter and spring frost. In France, for example, its cultivation is recommended only in the regions south of Bordeaux. Only few of these Japanese plums are pure *P. salicina*; most of them are hybrids, primarily with different native American plum species like *P. americana*, *P. angustifolia* and others, starting with the breeding work of Luther Burbank. All cultivars of Japanese plums are diploid ($2n = 2\times = 16$); most of them are self-sterile. Self-fertility was found to be an exceptional phenomenon among them (Alderman and Angelo 1933). Nowadays, some new varieties were released which are self-fertile or partially self-fertile (Ramming 2006). Self-fertility is supposed to be determined by the so-called S_e-allele, which seems to be identical with the S_5-allele (Pedryc et al. 2006).

The fruits of the present varieties are mostly big and round or heart shaped, but never oval or elongated. They are of attractive appearance and well suited for transport. For these reasons, there is an increasing demand in the market. The fruits are juicy, in most cases clingstone and have usually a much lower sugar and acid content than highly developed cultivars of the European plum, which are richer in aromatic compounds as well.

2.2.2 Apricot Plum (*Prunus simonii*)

No wild form of this species is known. It is cultivated in China, Japan and Central Asia since a long time and was described for the first time in 1872 (Kovalev 1941). The fruits are oblate and small with a diameter of 25–30 mm, dark to purple red. The flesh is firm, aromatic and clingstone. Its botanical position is unclear, and often it is considered to be an apricot–plum hybrid. Because of its firm flesh and strong flavour, it was used in Californian breeding programmes for the so-called Californian Japanese plum cultivars, for example, 'Shiro' and 'Wickson' (Teskey and Shoemaker 1978).

2.3 Wild Plums

Wild plums have been of special interest in variety and rootstock breeding as donors of resistance or ecological adaptability (Paunovic 1988; Surányi 1998). The most interesting species are described below.

2.3.1 European Wild Plums

P. cerasifera shows the greatest diversity of all *Prunus* species both in morphology and in ecological adaptation. The highest incidence the centre in nature and of diversity were found near the coast of the Caspian Sea. The fruits found there vary in diameter from 17 to 37 mm and the fruit shape from elongated to nearly

round (Kovalev 1939). The species occurs in this region up to 1800 m above sea level, adapted to widely different types of climate and growth conditions.

P. cerasifera, also called cherry plum, is diploid ($2n = 2\times = 16$) and of interest because of its good productivity, resistance to diseases, drought and heat, its early maturity and tolerance to unfavourable conditions. The cherry plum has small round fruits, with a diameter of 15–20 mm. Fruits of selected and cultivated types are bigger; some may reach nearly 40 mm. The fruit weight is between 9 and 15 g; some Iranian, Caspian and Georgian varieties weigh up to 25–30 g (Eremin 1978). The fruit colour ranges from yellow to red and dark violet. Most of them are yellow and then the fruits are frequently confused with mirabelles, but the fruit quality of myrobalan is much lower. The flesh is soft, juicy, sweet to sub-acid and of poor quality. The skin is tenacious and acid, and the stone is cling to semi-free. The fruits are not very suitable for fresh consumption, but favoured because of their early ripening. In Turkey, the fruits are eaten unripely strewed with salt.

The blossoming time is very early, about 2 weeks before that of European plum, Therefore, myrobalanes are very sensitive to spring frost. Ripening time is from June to September (Eremin 1978). In Germany, a type was found ripening at the beginning of October. In continental climate, the winter hardiness is very high up to -40°C. However, the lower chilling requirements cause problems in regions with fluctuating winter temperatures. Both types with winter hardiness and with heat resistance were selected as well as genotypes with high wilting point in the leaves (Eremin 1978). The myrobalan is of great interest for breeding purposes in regions with extreme climatic conditions. In Russia, interesting hybrids with *P. salicina* were made (Eremin 1978). In fruit growing, myrobalanes are mostly used as seedling rootstock. Trees grafted on this rootstock show strong vegetative growth and usually produce stem, but no root suckers.

For a long time, *Prunus insititia (P. domestica* spp. *insititia)* has been regarded as an own species; today it is classified as a subspecies of *P. domestica*. Members of the subspecies are used as rootstocks. They are of interest for hybridisation in rootstock breeding. The subspecies was also used in Russian breeding programmes to get varieties with better winter hardiness (Yenikeyev 1978). It can be divided into three groups (st.Julien A, Damson and Bullace). A differentiation between these groups is not always easy as natural hybridisation occurs.

In 1754, Miller described a plum called 'St. Julien' (Hedrick 1911). Its rootstock characteristics were discussed throughout the old literature (Faust and Surányi 1999). Whether the name 'St. Julien' was ever applied to a special cultivar is unknown. After studying the 'St. Julien plum' over 40 years, Küppers (1976) came to the conclusion that this plum type is ranging in appearance from small fruited plums as 'St. Julien A', with green-yellow fruit colour, to wild bullaces like the rootstock 'GF 655/2' with black fruits. For a long time, seedlings have been used as rootstocks. Different types of 'St. Julien plum'

were selected in East Malling and propagated vegetatively. The most important are 'St. Julien A' and 'Pixy'. From INRA Bordeaux, 'St. Julien GF 655/2' was selected, a rootstock commonly used in middle Europe, well adapted, but developing a lot of root and stem suckers.

Damson is a group having oval to roundish fruits, with a dark blue to green skin and a bitter, spicy and sweet taste; they are aromatic and astringent. In France, it is called 'Damas' and is used as rootstock, for example, 'Damas 1869', but today it is no longer recommended for use due to its tendency to sucker. In Germany, Switzerland and Austria, it is known as 'Krieche' with black fruits and 'Zibarte' with green-yellow fruits. 'Zibarte' is cultivated for brandy production and gives a high-priced brandy with special taste and aroma.

Bullace is yet another type of small, round fruits, which are usually dark blue and sweet. They are used for brandy production. It is called 'Haferpflaume' in Germany.

P. spinosa (blackthorn or sloe), yet another species, is widespread in Europe, North Africa and Northern Turkey, but also found in North America imported from Europe. The sloe is tetraploid ($2n = 4\times = 32$). One of the centres of origin is the Caucasian region where types of *P. spinosa* with chromosome sets of $2n =$ 16, 24, 32, 40, 48, 64 and 96 were found (Zuhary 1992). The species is growing in glades, forest borders, river valleys and mountain slopes. It is characterised by a broad adaptability and good viability. It survives on dry soils and often grows on eroded soils and stony slopes. It is a strongly branching shrub, but can also give trees reaching a height of 4–6 m. The young plants are very thorny, whereas old plants are usually free from thorns. Flowers are arranged as single flowers, and the blooming period is early in the spring before the leaves emerge. The fruits are small with a mass of 2–5 g, mostly clingstone with tart flesh and very astringent, sweet only after frost impact. There are some reports about sloes with sweet fruits, but these are bullaces and not sloes. Blackthorn is very drought resistant and often used for stabilising stony slopes.

The sloe is used as dwarfing rootstock, but there are problems with suckering. Because of its dwarfism and resistance traits, it is used as crossing partner in rootstock breeding programmes.

2.3.2 American Wild Plum Species

There are many wild plum species present in North America. More than 20 species are known. Several native plum species have been characterised concerning their resistance traits (Beckman and Okie 1994). Some of the most interesting species will be described as given below. More detailed information is given by Rehder (1954) and Ramming and Cociu (1991).

At the beginning of the 20th century, clones with higher fruit quality were selected from native plum species. The names and years of their introduction are given by Faust and Surányi (1999). Unfortunately, these cultivars disappeared with the introduction of Japanese and European plum cultivars. Selected native North American *Prunus* species and their use are described by Beckman and Okie (1994).

P. americana is the most common wild plum with a small and usually thorny, spreading tree. Its native area of circulation reaches from Massachusetts to the Gulf of Mexico and to New Mexico (Faust and Surányi 1999). Mostly, fruits are red with a cling or free stone and an astringent skin. The flowering time is late. It is a donor of cold hardiness, but suckering is a big problem when used as rootstock. *P. americana* is the most common native species used in North American Japanese plum breeding programmes (Ramming and Cociu 1991). Hybridisations with *P. domestica* are rarely successful.

P. angustifolia is the 'Chicksaw plum', native from Delaware to Florida and Texas and Southern Ohio. The small bushy tree is usually suckering. The fruit is small and cherry-like, bright to red coloured, sometimes also yellow. The species is abundant on sandy soil and has a low chilling requirement. It was successfully crossed with Japanese plum resulting in cultivars like 'Segunda', 'Robusta' and 'Byrongold'.

P. nigra is the Canadian wild plum having red-orange to yellowish fruits with an astringent skin. Because of its cold hardiness, it was used in breeding for northern plum cultivars (Oldén and Koch 1962). *P. subcordata* is the Sierra plum (also called Western or Pacific plum) and resembles more to the European and Asian species than other ones. It is a very good cropper. The clingstone fruits are round to oblong with dark red to purplish skin and with sub-acid flesh. Some cultivars have been developed (Ramming and Cociu 1991).

P. hortulana is the 'Hortulan plum' and is a relatively tall upright tree (5–10 m) with small (25 mm in diameter) and red- to yellow-coloured fruits. The flesh is acid and clingstone with best fruit quality of all native North American *Prunus* species. The species is of some interest in rootstock breeding because it is dwarfing, not suckering and compatible to plum and peach.

P. munsoniana is known as 'Wild Goose plum' and is native in the regions of Kentucky, Texas, Tennessee and Kansas. The botanical status of this group is uncertain. This species represents a range of forms separated from the old *Hortulana* group. Trees are 6–8 m high. The fruits are oval and bright, red and yellow coloured, with juicy flesh. Several varieties were cultivated but are not available today (Ramming and Cociu 1991). The Marianna plum is a hybrid of *P. munsoniana* × *P. cerasifera* it is used as a rootstock (Faust and Suranyi 1999).

3 Breeding Methods

3.1 Intraspecific Hybridisation

3.1.1 Blooming Time

Plums, especially Japanese plum cultivars, are flowering very early in the season. Blooming time depends not only on the species but also on the variety. In European plum, Szabó (1989) observed an average interval of 8 days between

Table 6 Blooming time of some European plum cultivars

Very early	Early	Medium	Late	Very late
Czernowitzer	Avalon	Bühler	Anna Späth	Blue Bell
Lützelsachser	Čačanska najbolja	Čačanska lepotica	Auerbacher	Italian Prune
Wilhelmine Späth	Čačanska rana	Čačanska rodna	Čačanska late	Pitestean
Zwintschers Frühe	Dabrowice	Ersinger	Carpatin	
	Haroma	Excalibur	Centenar	
	Jojo	Hanita	Elena	
	Jubileum	Hanka	Gabrowska	
	Opal	Katinka	German Prune	
	Ortenauer	Top	Harbella	
	Presenta	Top 2000	Herman	
	President	Topfive	Mirabelle	
	Ruth Gerstetter	Topking	Stanctus Hubertus	
	Tegera	Topper	Stanley	
	Tipala		Tophit	
	Valor		Tuleu Gras	
			Valjevka	

the time of full bloom of the earliest and the latest flowering varieties in Hungary. However, the blooming time depends also on the region. In warmer regions, the time span between the full bloom of early and late blooming genotypes is more prolonged than in cooler or in continental climate. Nicotra et al. (1983) report that in Italy, the variety 'Valor' started blooming 22 days before 'Jefferson'. The blooming times of important Euopean plum varieties are given in Table 6.

The blooming time of the individual flower depends on the position of the flower bud on the tree. Flower buds develop in lateral position on long or short shoots which always are on year old. Unlike most of the older varieties of European plum, new varieties usually set flower buds on long shoots. These flower buds are 2 or 3 days delayed in blooming time compared to the flowers developing on short shoots. The Japanese plums generally set a lot of flower buds on long shoots. The delay in blooming time on long shoot flowers ensures a better fruit set in case of bad weather conditions of individual days during the blooming period. However, fruits developing from flowers on long shoots are ripening a bit later than the other ones.

The length of the flowering period is genetically determined but largely modified by the environment as well. Szabó (1989) divides the varieties into three groups depending on the length of their flowering time: short (less than 8 days), intermediate (8–11 days) and long (more than 11 days). The flowering period of Japanese plum is generally shorter than that of European plum.

3.1.2 Fertility

Fertility is expressed as the percentage of number of fruit developing out of a known number of flowers. It genetically determined. Most of the varieties of *P. salicina* and all American species as well as their intraspecific hybrids are considered for practical use as self-sterile. In recent years, but there are some new fertile or partially self-fertile Japanese plum cultivars introduced (Ramming 2006). *P. cerasifera,* also a diploid species, cannot be considered as entirely self-incompatible, because fruit set after self-pollination is low (Shoferistov 1986). In European plum, self-fertile, partially self-fertile and self-sterile genotypes are known (Table 7). The extent of self-fertility is a result of different external and internal factors and depends, to a high degree, on the flower quality as well as on the temperature. The temperature influences the speed of the pollen tube growth and the aging of the ovule (Figure 1).

Fertility tests are made by isolation of branches and self-crossing. Tests for partial self-fertility are made by comparing the fruit set after cross-pollination to that after self-pollination. For the assessment of the fertility of a respective genotype, investigations over a period of more than one year are necessary. Both pollination and fertilization are necessary for fruit set in plums. Parthenocarpy has never been observed under natural conditions.

According to their fruit set after open pollination, Szabó (1989) assigned 58 European plum varieties to four groups (Table 8). The fruit set that is optimum under practical conditions depends on the degree of the flower set and on the fruit size of the respective cultivar. It varies between 10% for genotypes with large and 20% for those with smaller fruits. After cross-pollination in the breeding process, the fruit set can be higher and may reach more than 50%.

In average, Japanese plums have a higher flower set and bigger fruits than European plum cultivars. A fruit set of 5–10% is enough to get a good yield. Szabó classified the varieties of the Japanese plum into three groups concerning

Table 7 Fertility of European plum cultivars

Self-fertile		Partial self-fertile	Self-sterile
Auerbacher	Katinka	Bluefre	Avalon
Bühler Frühzwetsche	Nancy Mirabelle	Čačanska rana	Excalibur
Čačanska lepotica	Presenta	Čačanska najbolja	Green Gage
Čačanska rodna	Stanley	Chrudimer	Lützelsachser
Elena	Tegera	Ersinger	Magna Glauca
German Prune	Top 2000	Italian Prune	Opal
Hanita	Hanka	Jubileum	Ruth Gerstetter
Harbella	Topfive	Ortenauer	President
Haroma	Topking	Tophit	Valor
Herman	Topper	Voyageur	Zimmers Früh-
Jojo	Valjevka		zwetsche

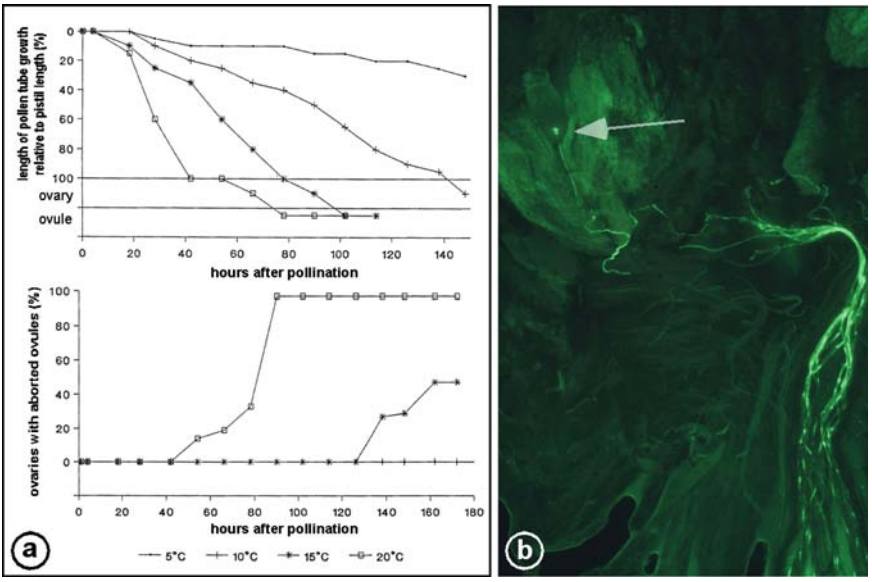

Fig. 1 Pollination and fertilisation in European plum. (**a**) Influence of the temperature on the pollen tube growth and the aging of ovules in the cultivar 'Lützelsachser' (Hartman and Stösser 1994). (**b**) Pollen tube growing through the micropyle into the embryo sac (arrow) in a plum ovule (pollen: 'Haganta', ovule: 'Pacific')

their fertility traits (Table 8). Especially in case of bad weather conditions during flowering, cross-pollination resulted in a higher fruit set even in self-fertile varieties.

3.1.3 Intersterility

Cross-incompatibility prevents fertilisation between special combinations of plum varieties. Among European plum cultivars, a low frequency of intersterility was found. Tehrani (1991) reports incompatibility between special varieties bred at Vineland Station in Ontario. This can be explained by their close

Table 8 Groups of self-compatibility and fruit set in plums varieties (according to Szabó 1989)

European plum Group	Fruit set	Frequency of varieties	Japanese plum Groups	Fruit set
Low	<10%	10.30%	Low	<5%
Intermediate	10–20%	22.40%	Intermediate	5–10%
High	20–40%	54.00%	High	>10%
Very high	>40%	10.30%		

relationship. Pollen tube growth is influenced by S-alleles. Sutherland et al. (2006) found 15 alleles for S-RNases in myrobalanes and 12 in *P. domestica*. Sequence comparisons of coding regions of plums and myrobalanes showed a high identity with published *Prunus*-S-RNase-alleles. The S-locus consists of two genes, the S-RNase gene and the SLF/SFB gene. The S-RNase is the female determinant. It is secreted in large amounts into the extracelluar matrix of the style. SLF/SFB, the male determinant, is a member of the F-box family proteins; it is responsible for the degradation of S-RNases in compatible pollen tubes (Takayama and Isogai 2005).

In several studies, intersterility with 'Italian Prune' was observed (Lee 1980; Tehrani 1972, 1991). However, this incompatibility is never absolute. The results can be explained by the sensitivity of the variety to low temperature. In the Hohenheim extensive breeding programme with crossings between a lot of European plum varieties and also in fertilisation studies (Hartmann and Stösser 1994), intersterility has never been observed. Szabó and Nyeki (2000) reported about the cross-fertility of some European and Japanese plums. The low frequency of intersterility among European plums can be explained with the hexaploidy of the species. In Japanese plum, intersterility occurs more often.

3.1.4 Sterility

A low fruit set may be the result of morphological sterility based on short style, small stigmata or underdeveloped ovary. This phenomenon can be observed more frequently in Japanese than in European plum (Bellini et al. 1996, Palara 1996). Over a period of more than 20 years, Surányi (1994) explored the flower anomalies of plums and found that the traits of sterility are inherent but that there are also seasonal effects. On young plum trees, more sterile pistils are found than on older trees. The low fertility of seedlings in the first or second year of flowering is based on the ontogenesis of the plant. It is a sign of the juvenility of the plant.

Male sterility is known in plums since Crane (1925) reported about this phenomenon in 'Gold Esperen'. The Romanian variety 'Tuleu Gras' is male sterile as well. This male sterility is inherited dominantly. All descendants of this variety obtained in the Hohenheim breeding programme were male sterile. Surprisingly, even some other parts of the flowers were degenerated in some descendants. Often, the petals were very small, green coloured and sometimes totally reduced. Fifteen varieties introduced in Romania are male sterile, and some of them are the most valuable ones (Botu et al. 2001).

3.1.5 Pollination

Pollination is the transfer of the pollen to the stigma. In cross-breeding, this is only possible with varieties of nearly the same blossom time. Stösser (1985) found

a decline in the fruit set when pollination was made after the fifth day of flower opening. In our experience, the best time for pollination is the first 2 days of the opening of the flower. If there is a requirement of crossing between varieties with larger differences in blooming time, there are several possibilities to make a pollination possible: one can use trees growing in different regions with different climatic conditions, branches of the male variety can be cut and put in a warm house the pollen of the earlier blooming variety is stored in a refrigerator.

There is no loss of viability during the storage of pollen at 4°C for a period of 8 days. Lorenz (2000) found a decline of 30% in pollen germination after a storage time of 2–3 weeks at 4°C. Using pollen stored in evacuated glass tubes at –1°C to –20°C for 1 year, Lee et al. (1981) observed good pollen tube growth. This may be an interesting method for pollen conservation, as Anvari (2006) obtained a good fertility after the pollination of apple flowers with pollen stored, using this method, for more than 12 years at −20°C.

Both in European and in Japanese plum, the results of cross-pollination depend much more on the female parent than on the quality of the pollinator. Good pollinators within the European plum are, for example, the cultivars 'Stanley' and 'Čačanska lepotica'. Good pollinators produce about 50 000 pollen grains per flower. In 'Stanley' and 'Italian Prune', more than 70 000 were found (Hartmann and Stösser 1994).

In crossing experiments, the quality of the pollinator must be considered. The quality of the pollen depends on the deposition of starch. The highest content was found just before the opening of the flower; high starch content in the pollen grain correlates with the speed of the pollen tube growth (Lorenz 2000). Therefore, for collecting pollen for use in crossing, flowers of the male parent should be picked just before opening (in the so-called balloon stage). The best time for the pollination is 1–3 days after the opening of the flower. In this case, flowers of self-fertile genotypes must be emasculated before the pollen is ejected. Under field conditions, emasculation (Fig. 2a) results generally in a

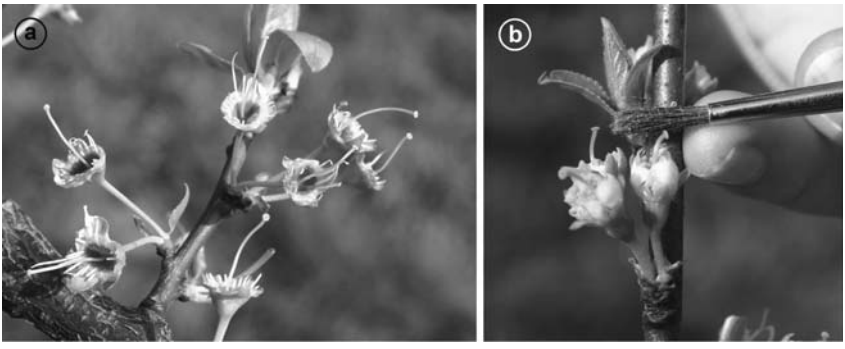

Fig. 2 Pollination in plum breeding. (**a**) Emasculated plum flowers ready for pollination. (**b**) Pollination of flowers in the balloon stage after having removed most parts of the petals

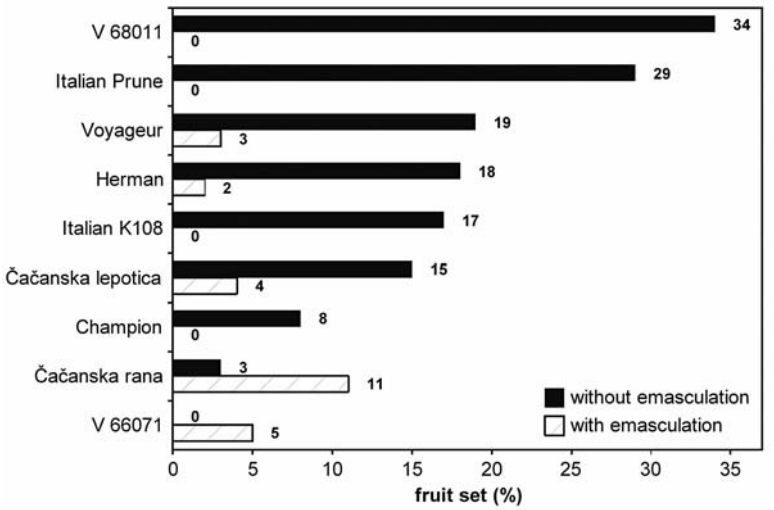

Fig. 3 Fruit set after self-pollination in different European plum cultivars (Kellerhals and Rusterholz 1994, modified)

poorer fruit set (Fig. 3). This is also the case when the climatic conditions are in optimum (Kellerhals and Rusterholz 1994).

Therefore, a method was developed to help to avoid the necessity for emasculation: petals of flowers in the balloon stage are removed 1 or 2 days before the opening of the flower. At this phase of flower development, a self-pollination is not possible but the stigmata are already receptive for foreign pollen. The pollen is transferred to the stigma using a fine brush (Fig. 2b). After the pollination, the branches with the pollinated flowers must be isolated in order to avoid the uncontrolled pollination by insects. Bags of synthetic material with a diameter of 20–30 cm and a length of 40–50 cm can be recommended. The duration of flower isolation depends on the weather conditions and should be at least 1 or up to 2 weeks.

3.1.6 Germination

A stratification of the seeds at 4–5°C for 3–4 months is necessary because their dormancy has to be overcome. Stratifying more than 10 000 seeds directly after harvesting, Jakubowsky (1998) achieved an average germination rate of 33% on the average of 6 years. The annual fluctuation was very high: while 59% germinated in 1996, only 20% germinated in 1991. A main problem in the germination process is the thickness of the stones. The germination of some seeds may be delayed for 1 or 2 years. Paunovic et al. (1968) obtained 976 seedlings out of 4 284 seeds (22.8%), and only 11.3% reached the adult phase.

As the costs for pollination are very high in *Prunus* species, such low germination rates are not satisfying.

Theiler (1971) developed a special method of embryo culture for cherries. This method was successfully used for plums and prunes (Hartmann 1994). Stones are carefully cracked using a bench vice. For swelling, the seeds are incubated in a fungicide solution over night. The testa and residues of the endosperm adhering at the embryo must be removed using pincers or fingernails (Fig. 4a). The embryos are sown in a sterile substratum containing peat, sand, perlite and/or vermiculite. For optimum growth of the seedlings, temperatures of 25°C for 16 h during the day and 15°C during the night are recommended. The germination step should be done in a climatic chamber, but a heated greenhouse with additional light can be used as well. The application of fungicides may be necessary to prevent fungal infections of the young embryo. Within a week, the cotyledons get green and the radicle starts growing. About 3 weeks after sowing, the young plants are transplanted into bigger pots and transferred to a greenhouse. Using this method, the germination rate is very high (up to 90%). The germination of the embryos can be started immediately after harvesting the fruits without the need for stratification. Under good cultivation conditions using additional light for enhancing the plant growth, the young seedlings can reach a height of 150 cm till the end of the year of harvesting the fruits. The method of embryo culture is time consuming. Therefore, this method is applied mostly in winter times starting at the end of January. Until that time, the stones are stored under dry conditions at about 10°C.

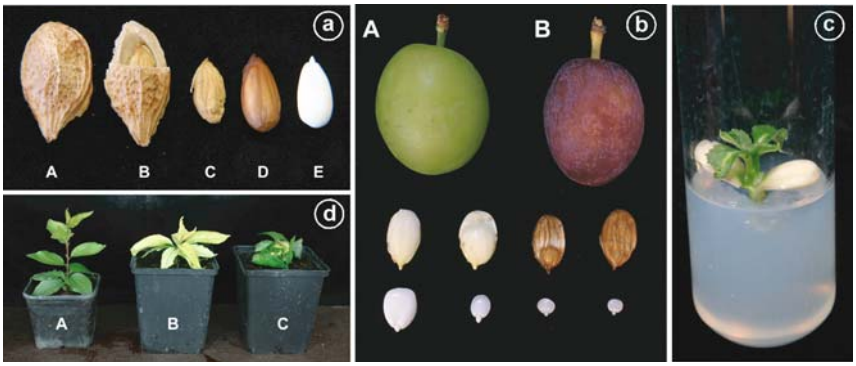

Fig. 4 Germination and growing of plum seedlings. (**a**) Preparation of embryos: A stone, B cracked stone, C seed, D seed after water uptake (swelling), E embryo (seed after removal of testa an endosperm residues). (**b**) Underdeveloped embryos in early-ripening cultivars ('Ruth Gerstetter'): upper row, fruits; second row, seeds; third row, embryos. A: normal fruit, 11 weeks after full bloom. B: fruit that will undergo premature fruit fall. Even a big part of normally developed fruits contain underdeveloped embryos. (**c**) Underdeveloped embryo of an early-ripening cultivar starts growing in vitro (embryo rescue). (**d**) Effects of inbreeding ('Ortenauer' × 'Ortenauer'): A, normally developed seedling; B, seedling with chlorophyll deficiency; C, seedling with genetically determined dwarfism (*See* color insert)

There are also some other germination techniques, for example, the 'hot chilling' method: Tehrani (1991) obtained quite high germination rates when keeping the seeds for a time period of 3 weeks at 21°C and, afterwards, at 5°C. Germination started 3 months later. In the Weihenstephan breeding programme, high germination rates were obtained with the following method for sawing in vitro: the stones are cracked and the seeds are soaked in tap water until they are swollen. Afterwards, they are surface sterilised (20 min in 1.5% NaOCl plus small amounts of Tween® 20) and put into an MS-Medium supplemented with 1.44 µM 6-benzylaminopurine. They are stored at 4 °C in a cool chamber in the darkness. After 12-40 weeks, the embryos start growing. The young plants are adapted to soil conditions in the greenhouse after the radicule has reached the length of 0,5-1,0 cm.

The seeds of early-ripening varieties are often imperfectly developed (Fig. 4b). Therefore, the germination rate is usually very low. In vitro embryo culture (Fig. 4c) was applied successfully by Bellini and Nencetti (1998). Gerecheva and Zhivondov (2002) describe an embryo rescue method. In experiments with the cultivar 'Burmosa' (*P. salicina*), the germination rate was 70–100%. The adaptation to the medium composition and the culture conditions is necessary. The smaller the embryos, the higher the demand of the composition of the culture media (Ramming 1990). Embryo rescue techniques are also applied to seeds obtained from intraspecific crossings in case the seeds are not fully developed. At Weihenstephan, good results were obtained when immature embryos were cultivated on c_2d-medium described by Chée and Pool (1987).

3.1.7 Cultivation of Seedlings

Seedlings obtained using the embryo culture method avoiding stratification as described above may stop growing after 4–6 weeks. After spraying gibberellic acid (GA_3, 0.5 g/L, in 50% (v/v) ethanol), the terminal bud starts growing again. Additional light during the cultivation is very useful for a good development. Alternatively, the seedlings can also be exposed to light for 24 h per day, and then the application of gibberellic acid is usually not necessary.

When the seedlings have reached a height of more than 50 cm, they can be planted directly in the field; otherwise one year of cultivation in the nursery is recommendable. Good horticultural practice (fertilisation, irrigation, pesticide/herbicide treatments, etc.) should be applied during the following years in order to enhance the vegetative growth of the seedlings. In this way, juvenile period can be overcome as soon as possible. Attention should be paid to aphid and especially mite control. In some years, the vegetative growth is strongly reduced by the mite species *Aculus fockeui*.

The seedlings are planted in the field at a distance of about 4 × 1.25 m. The better the seedlings grow, the earlier the first flowers appear. Depending on the crossing combination, individual seedling may flower as early as in the second

year. The majority of seedlings will remain in the juvenile phase for about 4 years. Therefore, the grafting of seedling budsticks on dwarfing rootstocks as recommended in apples or pears for earlier flowering is not necessary.

Sporadically, genetic dwarfism, chlorophyll deficiency and albinism or fasciated growth forms can be observed. Such genetic defects occur much more frequently after self-pollination (inbreeding depression, Fig. 4d). Mostly, these seedlings are of low vitality and rarely bear fruits in case they survive during the first years at all. Therefore, they can be discarded before planting into the field.

3.2 Interspecific Hybridisation

P. domestica itself is considered to be a hybrid between *P. cerasifera* and *P. spinosa*. However, this hypothesis is often challenged. The botanical systematic of the genus *Prunus* is complicated and unclear. Nevertheless, so-called interspecific hybrids are of importance in plum breeding. For the improvement of rootstocks, methods of interspecific hybridisation between different species of the genus *Prunus* are commonly used. For example, the rootstock 'Marianna' is an interspecific hybrid between *P. cerasifera* and *P. munsoniana* (for more examples, see Chapter 6). For scion breeding, the impact of interspecific hybrids is, up to now, comparatively low. Only some hybrids between *P. salicina* and *P. armeniaca*, known as plumcots, are of commercial interest. Any interspecific hybrids between any species of plum and the apricot are called plumcots. Most of the existing plumcots are hybrids of *P. salicina* or *P. cerasifera* with apricots (*P. armeniaca* or *P. mume*) (Okie 2005). Okie (2005) gives a short overview of the history of plumcots. Ramming and Cociu (1991) give a detailed report on the genetic resources of plums including a description of the different species.

Interspecific hybridisations enable the possibility of transferring important traits, which only occur in one species to another one. For example, the cold hardiness of *P. spinosa*, *P. cerasifera*, *P. americana* and *P. ussuriensis* might be transferred to *P. salicina* or *P. domestica*. The high fruit quality of *P. domestica*, which is manifested in its high contents of organic acids, sugars and aromatic compounds, makes it a promising crossing partner for improving the poorer fruit quality of other *Prunus* species. Moreover, the European plum is the only *Prunus* species with genotypes completely resistant to the PPV mediated by hypersensitive response. Therefore, it is an interesting crossing partner for introducing hypersensitivity against PPV into other *Prunus* species. Recently, a breeding programme with this aim was started at Technical University of Munich in Weihenstephan. Genotypes of *P. salicina* excel other species in its fruit size and good transport and storage ability of the fruits. Thus, hybrids between European and Japanese plum seem to be promising in improving the pomological value of both species. Oldén (1965) reports on such hybrids. His

findings indicate that it is better to use the European plum as female parent because the fruit set and the embryo quality are much lower in the reciprocal combinations. Self-fertile genotypes of the European plum tend to give higher fruit set than self-incompatible genotypes when hybridised with *P. salicina*. However, there is a specific combining ability for the different genotypes of European and Japanese plum. The fruit set varied in between 0.0 and 19.4%.

The number of chromosomes varies within the genus *Prunus*. The European plum (*P. domestica* including *P. domestica* spp. *insititia*) is hexaploid ($2n = 2\times = 48$), the sloe tetraploid ($2n = 4\times = 32$), whereas the Japanese plum as well as most of the other *Prunus* species belonging to the group of plums are diploid ($2n = 2\times = 16$). Therefore, the chromosome status has to be considered in interspecific hybrids. Detailed investigations concerning this problem have been carried out by Oldén (1965). He found that seedling originating from crosses between hexaploid and diploid species usually showed 32 chromosomes (tetraploid), but betimes, hexaploid, pentaploid and octoploid seedlings occurred. In general, the vegetative characters of hybrids between *P. domestica* and diploid *Prunus* species were similar to *P. domestica*, whereas 'the flowers, their arrangement and the fruit characters were intermeditate or preponderant to the diploid parent'. The fertility of the hybrids was good. This example shows that interspecific hybridisation can successfully be used in breeding programmes.

In most cases, hybrids show leaf and fruit characters intermediate to those of the parents. Figure 5a gives an impression of leaf characters of parents and their interspecific hybrids. Although its chromosomes being very small, the hybrid nature of seedlings with *P. domestica* as one parent can easily be shown by counting the chromosome number in the root tips of the seedlings and determining in that way the degree of ploidy (Fig. 5b).

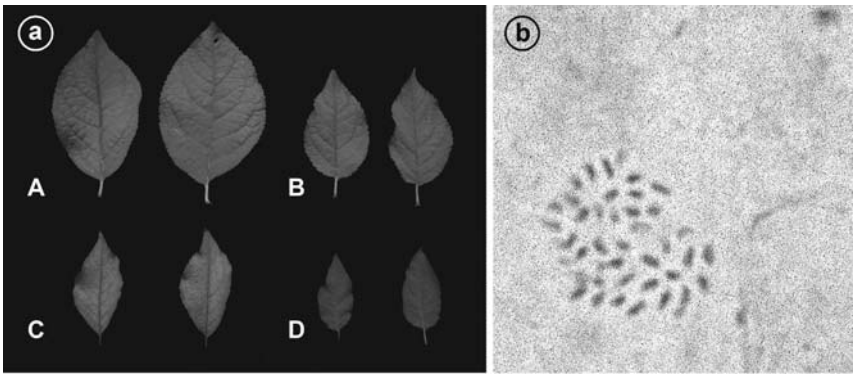

Fig. 5 Interspecific hybrids. (**a**) Leaves of *P. domestica* (A), *P. cerasifera* (D) and their interspecific hybrids (B, C), which show intermediate behaviour in size and shape. (**b**) Chromosomes of a pentaploid hybrid ($2n = 5\times = 40$) between *P. domestica* and *P. spinosa*. Roottips, stained with toluidine blue

3.3 Mutation Induction and Genetic Engineering

Most of the efforts in inducing mutations in stone fruit crops was made on peach, and only very few on plum (Srinivasan et al. 2005). Johannson and Oldén (1962) describe suitable methods for inducing polyploids, especially for the generation of unreduced gametes during meiosis using cholchizine and other mutagenic substances or irradiation. As mutations are often unstable in somatic tissue and tend to result in chimeric plants, they prefer to induce mutagenesis during the development of gametes and use them for breeding purposes. In some cases, for example for the generation of fertile pollen of triploid genotypes, they obtained good results with the colchizine treatment. There are some reports on the induction of mutagenesis in European plum using x-rays in order to obtain spur types of some plum cultivars (Cociu et al. 1997). Nowadays, the induction of point mutations, nucleotide insertions or deletions plays no role in plum breeding as it is not expected that important breeding aims can be achieved by single small-scale mutations of existing cultivars.

No reports are known concerning the somatic embryogenesis or the somatic hybridisation within the plum species. Despite the large efforts, genetic transformation and regeneration in plum have only been successful in single cases: a part of the coat protein gene of the PPV was transferred to the genome of seedlings of the *P. domestica* genotype 'B69158' (Scorza et al. 1994). One of these genetically modified seedlings, the clone 'C5', shows a level of resistance to PPV similar to that of the well-known quantitative resistant varieties of European plum (e.g.,'Čačanska najbolja'). It is assumed that the resistance of 'C5' is based on post-transcriptional gene silencing (Ravelonandro et al. 1998; Hily et al. 2004). However, this kind of resistance is not advantageous for the known quantitative resistance in existing cultivars as the genetically modified plants can get infected with the virus and can serve as host of PPV. New approaches are needed to resolve the Sharka problem. The bottleneck in the production of genetically modified woody plants is the regeneration of whole plants out of transformed undifferentiated tissue (Petri and Burgos 2005). Up to now, an efficient rate of transformation and regeneration in *Prunus* species was only achieved when seedling populations were used as base material (López-Moya et al. 2000; Mante et al. 1991; Srinivasan et al. 2005). López-Moya et al. (2000) express the opinion that for the moment, biotechnological methods cannot contribute to the improvement of plum cultivars concerning the PPV resistance. Currently, other characteristics of plum varieties are not tried to be genetically modified. Petri and Burgos (2005) consider genetic modification to have a certain value in the amelioration of fruit trees; however, they think that this method will not be applicable during the next time. The prerequisite for its successful use would be the development of an efficient transformation and regeneration protocol for a broad range of genotypes of both European and Japanese plum. Moreover, there is too less knowledge of the genetic

determination of agronomic important traits of plums, which is necessary for the successful application of gene transfer in practical breeding. Probably, gene transfer will be mostly restricted to plants used for scientific purposes. In this application, it can serve to understand underlying reasons for physiological processes. At the current state of knowledge, genetically modified plum varieties are not necessary for the plum production. Classical breeding methods are far from being the limit of the improvement of plum genotypes.

3.4 Selection Methods

3.4.1 Selection of Seedlings

Pre-selection. The long juvenile period is the major problem in breeding tree crops. A pre-selection to reduce the size of the seedling population is recommended. The degree of thorniness on young seedlings is regarded as a degree of wildness in many fruit tree species. A negative correlation between the thorns and the appearance of the first flowers was noticed for pears, as described by Stolle (1964). Thorny seedlings were, therefore, eliminated in plum progenies in most breeding programmes in the past, though van Mons already mentioned in 1835 that the appearance of thorns might be considered as a positive trait (see Loewel et al. 1957). Hartmann and Engelhorn (1992) found a positive correlation between thorniness in 2–3-year-old seedlings and the overcome of juvenility as well. Thorns are a sign of juvenility and can be found in seedlings from starting of the second year of cultivation. The earlier they are developed, the earlier the juvenility is overcome. The tree height and the stem circumference, which reflect the strength of the vegetative growth of a tree, correlate with precocity and, as it is the same with the size of the leaves, with the fruit weight. A pre-selection for resistance to PPV mediated by hypersensitivity is possible using the double-grafting method. However, a pre-selection for other characters is difficult and restricted to some attributes. Only seedlings with very little vegetative growth can be singled out without the danger of discarding valuable seedlings.

Primary selection. The primary selection is made in the field where the seedlings are growing. The blossom time is estimated using the different stages described by Berning et al. (1987). The flower set and the fruit set are rated on a scale ranging from 1 (no flowers/no fruits) to 9 (many flowers/overbearing). In this way, the yield potential of the individual genotypes can be determined. The sensitivity to diseases is estimated in a scale from 1 to 9 as well. Fruit characteristics are registered after picking samples of at least 30 fruits per tree. The fruit mass is measured determining the weight of the fruits. The soluble solid content is measured using a refractometer. Records of the fruit shape, colour of the fruit and the fruit flesh, the stone adherence, the juiciness, the taste and the aroma are necessary. The firmness of the fruits should also be registered. An

assessment of the firmness is sufficient because an exact measuring is very difficult. In the primary selection, a minimum of 3 years of evaluation with full crop of the individual seedling is recommended. The selection is made taking into account the breeding aims. The most promising seedlings are propagated for a further selection step in different regions and also for a test of their Sharka resistance.

Second selection. Plums and prunes are very sensitive to weather and climatic conditions especially during the flowering time. A transfer of the results obtained in one region to another is difficult. Therefore, the second selection should be carried out in different growing regions simultaneously. Four grafted trees of each seedling are sufficient. The measuring of the yield (in kg per tree) is useful, but the estimation in a scale ranging from 1 to 9 is sufficient. It is very important to compare the yield (and all the other traits of pomological importance) directly with one or even more well-known existing varieties.

The testing for Sharka tolerance is also necessary either by grafting on infected rootstocks or by inoculation of young trees with PPV-infected chips. At least three trees of each genotype to be tested should be artificially infected and, additionally, three virus-free trees should be planted into the PPV-testing orchard in order to test the field resistance as well. A monitoring should be done to estimate the leaf and the fruit symptoms and also a possible change in the ripening time. Moreover, the estimation of the yield is useful. In case of unclear PPV symptoms on the leaves, appropriate detection methods (e.g., ELISA, RT-PCR) should be used for the confirmation of a PPV infection. The testing should be carried out for at least four seasons until first conclusions are drawn.

In the past, it took about 20–25 years from the planting of the seedlings until the release of a new cultivar. Nowadays, this time has shortened to 10–15 years because of new procedures in handling the seeds, better selection methods and simultaneous testing in different regions.

3.4.2 Clonal Selection

Many important plum varieties have been in cultivation since centuries. In former times, plums were often not grafted on rootstocks but grown on their own roots. Root suckers of existing trees were used for vegetative propagation. Sometimes rootsuckers may have been confused with seedlings growing under the tree of interest. In this case, seedlings were used for propagation by an oversight; there was a new genotype spread under the name of the mother variety. In some cases, the seedlings will have been of better pomological value than the mother plant; in most cases, it has been vice versa. Moreover, mutations occurred during the long period of cultivation of old and widespread varieties. Therefore, the name of some of these varieties is, nowadays, rather a collective name than a true name for an individual genotype. Therefore, in these ‘cultivars’, a selection of the best genotypes might be useful. In all these cases, a clonal selection is essential. Mutations can affect all traits, but they can only be detected

when traits are modified, which can be distinguished easily, e.g., fruit colour, size, ripening time, etc. Bud sports or mutations have been found for variations in fruit and flesh colour, fruit shape and size, ripening time, yield and productivity.

The mutation rate can be increased using x-rays but also by heat therapy, which is used to produce virus free material. In Germany, for example, a virus-free clone of 'German Prune', called 'clone Rheinland', resulted from heat therapy and was propagated for some years in the nurseries. Each year, the trees of this clone had a good flower set but never set fruits. On an individual tree of 'German Prune, clone Rheinland', one branch with high annual crop load was found. In this way, the mutation character of the clone 'Rheinland' was shown.

Usually, mutations affect only one gene. However, sometimes also more characters are influenced. On the top of a tree of 'Bühler Frühzwetsche', for instance, Hartmann (1991) found branches with fruits ripening not only 2 weeks later but also being elongated in shape instead of round.

The first step in clonal selection is to start a general inquiry of fruit growers to call special outstanding trees. After extensive research on the desired traits, the best ones are singled out and propagated for planting in several locations for further selections.

There are some reports about selections of the most famous prune in Middle and Eastern Europe, the 'German Prune'. In the former Yugoslavia, selections were made by Paunovic and Gavrilovic (1978), in Germany by Hartmann (1983, 1986) and in the Czech Republic by Blazek (1991). In Poland, Rozpara et al. reported about a preliminary selection (1998a) and about a further selection of this clones (1998b). The most important trait in these selections was the fruit size. Hartmann (1986) found a variation between 18 and 28 g. There are also differences in vigour, yield, sugar content and ripening time. Also within the variety 'Bühler Frühzwetsche', clones were found showing differences in ripening time of three weeks (Hartmann 1989).

There are also some clonal selections of the cultivar 'Italian Prune'. Most important are those with a higher specific yield. Some clones were selected within the variety 'Mirabelle de Nancy' as well. The most interesting one is clone No. '1725' with large, pink spotted fruits. No. 'P 2778' is a clone of 'Mirabelle de Mete' with high sugar content and typical aroma. They are well suited for brandy production.

Nowadays, the impact of clonal selection in plum breeding is decreasing as more and more emphasis in placed on 'conventional' hybridisation breeding.

4 Breeding Objectives and Genetic Resources

Modern plum-breeding activities aim at the development of varieties that are adapted to different climates. They should grow successfully in specific localities and give attractive fruits with good quality for profitable marketing. Winter

hardiness for northern and lower chilling requirements for southern production areas are important breeding aims. Productivity and resistance, shipping ability and, especially for late-ripening varieties, long storage ability are of atmost importance. According to Weinberger (1975), twelve fruit characters are of major interest. However, these fruit traits vary from country to country.

Fruits of Japanese plums are primarily used for fresh consumption and for dessert. The shipping ability of the fruits is usually good. The fruits are quite attractive but their taste is, in most cases, dissatisfying.

European plum fruits are used for dessert and fresh consumption, and also for canning, processing, drying, cooking and in bakeries for producing plum cake. Some varieties, for example, 'Italian Prune' and also 'German Prune', can be used for all of these purposes. This kind of all-purpose varieties has been popular in Middle and Eastern Europe. All of them have got a small fruit size because big fruits cannot be used for drying, for instance. In the future, however, breeding programmes will aim to obtain genotypes with bigger fruits and excellent taste for the fresh market on the one hand and, on the other hand, varieties with smaller fruits which have firm flesh, are freestone and can be used for processing and in bakeries.

Special breeding programmes are necessary for the different purposes. One should take into consideration that the performance of a plum genotype depends, to a large extent, on the climatic conditions where it is grown. Therefore, a transfer of the results obtained in one region to others is not always possible and more difficult than in many other fruit species. Ideally, plum breeding is located in the main production area.

Because of a high degree of heterozygosity and, in case of *P. domestica*, its hexaploid nature, it is very difficult to investigate the inheritance of an individual trait in plum. Quantitatively and qualitatively expressed traits are known. The contribution of gene dose effects to the phenotypically visible characteristics of a trait has to be taken into account. Several studies were made concerning the inheritance of individual traits of interest. Most of these studies are found in the older literature. Inheritance studies are time consuming and require accurate planning, data collection and data interpretation. For many characters, such as disease resistance, a system for the classification of the genotypes in different classes has to be developed in advance. Appropriate statistical methods have to be applied. Often, the collected data are not normally distributed so that non-parametric statistical tests have to be used. If one of the mentioned points is not considered, the conclusions drawn in a study of inheritance are doubtful.

For inheritance studies, a large progeny per crossing combination is necessary. The more descendants are evaluated, the better are the conclusion that can be drawn. For practical use, about 100 seedlings may be enough. If just a tendency in inheritance has to be evaluated, 50 seedlings of one crossing combinations are usually sufficient. The size of the progenies is quite small

compared to that one usual in pome fruit breeding because it is much easier to get a large progeny in pome than in stone fruit breeding.

Genetics in European plum are better studied than in Japanese plum, but the knowledge is still insufficient in *P. domestica* as well. Donors for 24 traits are given by Cociu (1997b).

4.1 Climatic Adaptation

Plums growing in different areas and some of widespread cultivars such as 'Prune d'Agen', 'Italian Prune', 'Stanley' and 'German Prune' show a high adaptability to different climatic conditions. Nevertheless, in northern latitudes, the cultivation is restricted by climatic factors. Winter starts early and temperatures fall down to −25°C or even lower. Genotypes of *P. domestica* are generally more resistant to winter frost than that of *P. salicina*. Some varieties are able to withstand even temperatures below −30°C. Breeding for winter hardiness has been an important aim in some countries. A successful selection was made by Mitschurin in descendants derived from crossings of *P. domestica* with *P. besseyi* and *P. maritima*. *P. besseyi* was used as donor of frost resistance in North America as well. But only few hybrids survived and were mostly used as rootstocks (Okie 1995). Yenikeyev (1978) found the hardiest seedling for wood and flower buds in crossings with forms of *P. insititia*. In Russia, varieties of *P. domestica* like 'Vengerka Moskovskaya', 'Zuysinskaya' and 'Reine Claude Reform' were used as donors of winter hardiness. Eremin set up a large breeding programme using intraspecific crossing in order to develop plums tolerant to winter coldness (Okie 1995). Cold resistance of seedlings from different intraspecific crossings was tested by Kolesnikova (1978). Doroshenko (1998) stated that the RNA/DNA ratio in apical buds of 1-year-old shoot can be used as criterion for pre-selection for resistance to early frost in the autumn and to recurrent frost in winter. The main index of cultivar resistance to recurrent frost is the increase of fructose content in buds in year-old shoots.

Fluctuating temperatures during winter often cause damages to trees of some plum cultivars. Trees of varieties developed in continental climate with high frost resistance may be damaged in more maritime areas because the dormancy is broken by changing temperatures. Stem bark and flower bud damages have been observed. Stem damages developed during winter may be dangerous because the damaged tissue can subsequently get infected with bacteria (e.g., *Pseudomonas* spp.) resulting in the dying off of plum trees, as it has been the case in some middle European countries during the last years. Frost damage of flower buds has been observed when a warm period in January was followed by very low temperatures in February. For instance, the varieties 'Čačanska lepotica' and 'Ruth Gerstetter' are very sensitive. 'Italian Prune' and 'German Prune' are known to be frost tolerant. Oldén and Koch (1962) claimed that winter hardiness is not associated with the frost tolerance of flowers in spring.

To a certain degree, the susceptibility to spring frost also depends on the genotype. However, the most important factor is the developing stage of the flower buds of the respective cultivar at the time where the frost event takes place as well as the quality of the flowers, which is mainly influenced by the height of the yield in the preceding year. Early flowering varieties are generally more exposed to spring frost. A direct comparison between the varieties is only possible when done in the same flowering stage. This complex system of influencing factors may be the reason why the degree of frost tolerance of respective varieties given in literature often strongly differs from experiment to experiment. Szabó (2002) lists the degree of frost resistance of many varieties. Local and old plum cultivars as well as primitive landraces may be donors of resistance to frost and drought (Paunovic 1988).

Genotypes of *P. salicina* are usually flowering very early in the season and are, therefore, more vulnerable to spring frost events. *P. besseyi* and *P. maritima,* later blooming than most European plums, can be used as donors of frost resistance.

Most varieties of *P. domestica* have a moderate to high chilling requirement. This is positive for frost resistance but gives problems in areas with low chilling. Even the chilling requirements of some Japanese plum cultivars are not fulfilled in subtropical areas. Inadequate chilling results in delayed and abnormal flowering and reduced yield. Cultivars with low chilling requirements allow the production in such areas and extend the early market season for 4 weeks (Sherman et al. 1992). Breeding programmes aiming at low chill cultivars are running in different countries especially in the southern hemisphere (Okie and Ramming 1999). At University of Florida, low chill germplasm of Japanese plum from Taiwan has been used. Two seedlings were selected with a requirement of only 100 chill units (Sherman et al. 1992).

Drought and heat resistance are important traits in areas with low precipitation and hot temperatures during summer. *P. microcarpa* is the most resistant species and can be used for interspecific hybridisation. Temperatures of more than 35°C can result in heat spots on fruits, visible as sunken areas, sometimes found in Japanese plum varieties but also in weakly coloured European plum cultivars like 'Jalomita' or 'Ersinger' and others. In blue-coloured fruits, the underlying may break down and darken. There are significant differences between the varieties.

Due to the change of climatic conditions during the last decade in Europe, an unusually high degree of twin fruit formation was observed. In some years, up to 50–80% of double fruits were observed depending on the variety. Twin fruits are not marketable. For cherries, it has been shown that high temperatures of more than 30°C during the flower bud formation are responsible for the development of twin fruits (Roversi et al. 2005). During the fruit development in twin plum fruits, often one of the twins is dying causing problems with subsequent *Monilinia* infections. The occurrence of twins largely depends on the variety. 'Stanley' and 'Čačanska lepotica' as well tend to form a lot of twins. This may indicate a correlation with high fruitfulness, but in 'Čačanska rodna' only some twins were observed. The tendency to form twins is inherited by 'Stanley' and 'Čačanska lepotica'.

4.2 Yield Potential

The yield of a stone fruit orchard is the result of several factors. The most important one is the sensitivity of the flowers of the variety to cool weather conditions at blossom time. For instance, 'Italian Prune' is very sensitive to bad weather conditions during flowering (Wahnelt et al. 1993) as well as 'Valjevka' and 'Čačanska najbolja', whereas 'Čačanska lepotica' and 'Katinka' are relatively robust. A delay of booming is desirable. There are some late-flowering varieties in *P. domestica*. The blooming time is the result of an additive gene effect (Hansche et al. 1975).

Today, growers only accept varieties with precocious, high and regular yield. Precocity is a problem in some older cultivars like 'Italian Prune', 'Bühler Frühzwetsche' and 'German Prune'. It is genetically determined in most of the newer varieties. Some of them can bear 5–10 kg fruits per tree in the second year after planting. A full crop is obtained in the third or fourth year with 30–40 kg/tree (30–40 t per ha).

Good donors for precocity and high yield are 'Stanley', 'Čačanska lepotica', 'Čačanska rodna' and 'Verity'. A marker for precocity and high yield is the development of flower buds on long shoots. In some varieties, the fruit set is too high, resulting in small, unattractive and tasteless fruits. A fruit thinning may be helpful. However, seedlings with tendency to over-cropping should be singled out during the selection process.

4.3 Ripening Time

In some countries, there is an extensive supply of plum in many years, resulting in low prices mostly at mid-season. A better price is usually realised at the end and especially at the beginning of the harvesting season. Therefore, an extension of the ripening time is desirable. In the northern hemisphere, fruits of *P. domestica* are harvested from the middle of June till the middle of October. *P. salicina* is picked from the beginning of June to the beginning of October.

Ideally, there should be at least one excellent variety for each week of the ripening period. This will result in 15–20 cultivars both for European and Japanese plum and for each purpose of use. The highest proportion of early-ripening descendants can be obtained crossing two early-ripening varieties. However, the embryos of early-ripening varieties are often underdeveloped or stones are even empty so that the number of seeds obtainable and their germination rate are very low. Thus, embryo-rescue methods are helpful. Alternatively, the early-ripening variety is used as male parent for pollinating a mid-season ripening mother variety. A transgression of the ripening time of the male parent variety is possible. For example the very early-ripening cultivar

Fig. 6 Late ripening and sharka tolerant varieties of high economic importance. (**a**) 'Presenta'. (**b**) 'Elena'

'Ruth Gerstetter' resulted from the crossing 'The Czar' and 'Bonne de Bry'. Both parents are ripening later in the season. The earliest ripening variety in European plum with quality is 'Ruth Gerstetter', the latest ripening 'Presenta' (Fig. 6). The breeding of late-ripening varieties is easier. Quite often, seedlings have a later ripening time than the parental varieties (Hartmann 1994) (Fig. 7). Studies on the inheritance of the ripening time were made by several breeders (Vitanov 1977; Paunovic et al. 1968. Cociu (1977) investigated 4450 plum hybrids—44% ripened earlier than the parents, 46% were intermediate and only 6% later ripening. According to Hansche et al. (1975), the ripening time is determined by several genes or alleles additive in effect. Fruits of young seedlings are generally ripening 4–8 days later than grafted trees of the same genotype.

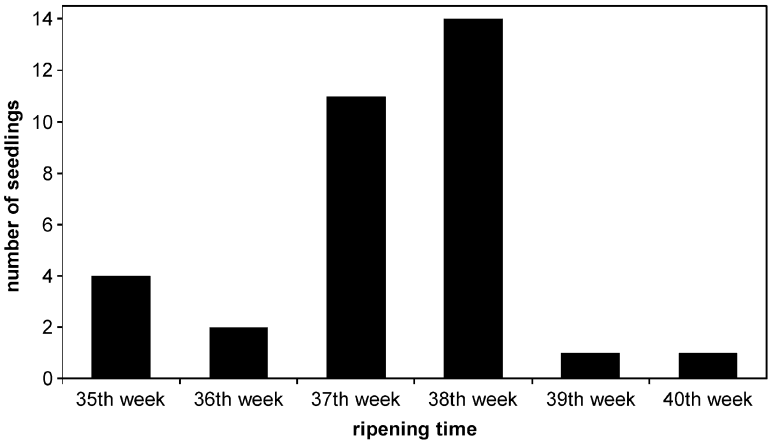

Fig. 7 Variation of ripening time in a progeny of European plum ('Ortenauer'×'Stanley'). 'Ortenauer' and 'Stanley' are ripening in week 36

4.4 Fruit Characters

The demands of the fruit vary from country to country and depend, to a high degree, on the predominating intended use. In some countries, there is a strict separation between varieties for fresh consumption on one hand and for processing on the other hand. There are also countries where people prefer all-purpose plum varieties, especially prunes, in Middle Europe. This may be due to historical reasons as the variety 'German Prune', the most widespread cultivated variety in these countries, is applicable for multipurpose use. It is one of the few varieties consumers know by name. Therefore, it is still very popular, and the market price is regularly higher than that of all other varieties.

4.4.1 Fruit Size

For fresh consumption, the fruit should outweigh 50 g. Most Japanese, but only some European plum cultivars reach this mass. Best known is 'President', but its taste is only medium, whereas the suitability for storage is good when the fruits are harvested not too late. Large-sized varieties attracting grower's attention are 'Jubileum' from Sweden and the newer German varieties 'Tophit' and 'Haganta' (Fig. 8). Some large-sized cultivars with good fruit quality were released from East Malling (UK) ('Avalon' and 'Excalibur'), but their productivity is low in case of suboptimal weather conditions during flowering. Sharka-resistant varieties with blue-coloured, large and firm fruits are missing.

For processing, the fruits should not exceed a mass of 40 g. Thus, the desired fruit size in Middle Europe and most Eastern European countries is 30–40 g. The fruits of the majority of recently released varieties are of this size. The fruit size is quantitatively inherited. A crossing of small-fruited varieties among themselves results in seedlings bearing small fruits. In case of both parents having large fruits, the progenies will mostly have smaller ones than their parents. Paunovic et al. (1968) found only 2.8% of all hybrids bearing fruits larger than the parents.

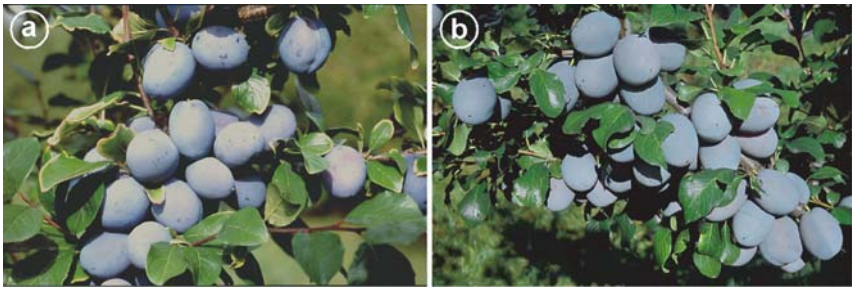

Fig. 8 Large-Sized varieties of European plum. (**a**) 'Tophit' (**b**) 'Haganta'

4.4.2 Fruit Shape

The fruit shape is not important in countries where people prefer large fruits for table use. On the contrary, the shape is very important in some other countries, for example, in Middle Europe. The fruits must be oblong to elongated like a prune because round fruits are regarded as plums, which are not popular in these regions.

In *P. domestica*, a wide range of different fruit shapes exists. Paunovic et al. (1968) report that this trait is quite constant—only 9% of the progenies show new kinds of shapes. This finding is contrary to the results of our breeding programme: very often, in an offspring of crossings between parents with elongated fruits, all different kind of fruit shapes can be observed.

4.4.3 Fruit Colour

The fruit colour of plums ranges from black to blue, purple, red and yellow. Some varieties are even bright coloured. The ground colour is often covered with waxy bloom, which makes the fruits very attractive. For fresh market, the fruit colour is an important trait. The preferred skin colour varies from country to country. In Middle Europe, blue-coloured fruits are preferred. Some varieties are coloured completely blue up to 3 weeks before ripening so that fruits are harvested unripe if the farmer is careless. Yellow fruits should be handled carefully as otherwise, some days later, brown spots may appear on the skin and the fruit attractiveness decreases rapidly.

Dark skin colour results from a high content of anthocyans and is inherited dominantly. As varieties with dark skin are often heterocygote concerning this trait, descendants originating from a crossing of two dark-skinned varieties can show red-, green- or yellow-coloured fruits (Fig. 9). Varieties with yellow skin colour are homocygote concerning this trait. Measuring the anthocyan content of fruits, Ogasanovic (1978) showed that in 36.1% of the progenies, the content

Fig. 9 Variability of fruit mass and colour in *P. domestica*. (**a**) Fruits of seedlings of one progeny ('Ort×Stan34' × 'Hanita). Different fruit sizes and colours. The fruit mass varies between 25 and 65 g. (**b**) Red- and yellow-coloured fruit of a seedling of 'Elena' × 'Ort×-Stan34'. Both the parents have blue-coloured fruits (*See* color insert)

was higher than that in their parents, indicating an additive effect of genes. An inheritance model of the fruit colour is given by Alehina (1978).

4.4.4 Flesh Colour

The colour of the flesh ranges from orange to yellow and greenish yellow to white. Red flesh is found only in Japanese plums and in cultivars of *P. cerasifera*. Flesh colour is more important for fresh consumption than for processing. An orange or golden flesh is preferred.

The flesh colour is a very variable trait. Paunovic et al. (1968) found that only 57% of the hybrids corresponded to their parents. In 43% of the seedlings, a new colour appeared. A good donor of orange flesh is the cultivar 'Hanita'.

4.4.5 Firmness

The texture of the flesh is the decisive factor for the firmness of the fruit. To a large extent, the firmness depends on the ripening stage of the fruit but also on the variety. It can be quantified using a penetrometer. However, the elasticity of a fruit, which correlates not per se with its firmness, can influence these data. For selection purposes in breeding programmes, an estimation using a five-group scale ranging from very firm to very soft is sufficient. The texture reaches from very fine to fibrous. In some varieties, the flesh is melting; in others it is mealy. A sufficient firmness is important for the transportation value. In some varieties, the firmness can be reduced very fast after harvesting, but this depends on the ripening stage.

Sometimes there is a relationship between the firmness of fruit and its juiciness. For fresh consumption, certain juiciness is desired. For use in bakeries, juicy and soft fruits cause problems (Fig. 9). Paunovic et al. (1968) found that firmness is the most variably inheritable character in plums. Seventy-five percent of the progenies were softer than the parents. Good donors for high firmness are 'Katinka', 'Tegera' and 'Čačanska lepotica'.

4.4.6 Stone Adherence

Generally, fruits which have a pit free from the flesh are desired not only for fresh consumption but, to an even larger extent, for using the fruits in bakeries and for processing.

There are different stages in stone adherence ranging from freestone to semi-free and clingstone. This shows the quantitative character of the trait, which may be determined by gene accumulation. Clingstone seems to be dominant over freshtone (Paunovic et al. 1968; Wellington 1927). Donors for freestone are 'Čačanska lepotica', 'Tegera' and 'Katinka' (Hartmann 2007). Within a certain genotype, the degree of stone adherence can vary from year to year.

4.4.7 Endocarp Splitting and Gummosis in the Fruit Flesh

During the last decade, endocarp splitting or shattering in fruits of *P. domestica* got more and more a severe problem which was unknown before. Sometimes there is no splitting of the stone (endocarp) but caverns are developing in the fruit flesh (mesocarp), and these caverns may be filled with gum. One reason for this may be the focus on larger fruit size during the selection, but caverns are also found in small fruits especially in early-ripening varieties. For example, in 2007 more than 90% of the fruits of the variety 'Herman' showed caverns in Germany. The rapid expansion of the distal part of the embryo cells and the possibly early hardening of the endocarp may be involved in the development of this phenomenon. Factors that enhance the fruit size and rapid changes in the weather conditions worsen this disorder. There are genetically determined differences between the varieties, but fundamental research on this subject is outstanding. In future breeding programmes, a low tendency to endocarp splitting and to gummosis in the fruit flesh should be considered as an important selection criterion.

4.4.8 Taste

The most important aspect of fruit quality is the taste. This fundamental aspect has to be considered by any breeder. The most attractive variety may be of interest for the moment but not during time if the requirements concerning the taste are not fulfilled. In case a variety is not tasty, the consumer will not buy it again. Consumes know very few about the specific quality of different varieties. Some kind of 'education' is necessary.

The preferred taste varies from consumer to consumer and from country to country. In South Europe and also in Asia, people prefer sweet fruits. In other countries, varieties holding a good balance between sugar and acid content are favoured. Fruits with an intensive flavour and firm fruits that soften prior to consumption are desired.

The fruit quality and the taste depend on the sugar content, usually estimated using a refractometer. The soluble solid content highly correlates with the sugar content. In plums, there is a wide range from 12 to 25% Brix. Prunes are higher in sugar content than plums, and late-ripening are higher than early-ripening varieties. In order to obtain a good fruit quality, a minimum of sugar content is necessary. Kadar (1999) reported that plums should have a minimum of 12% soluble solids. In late ripening varieties, the content of soluble solids should be more than 17% Brix. The perception of sweetness depends on the acid content. According to Vangdal and Flatland (2007), the ratio 'soluble solids–total titriable acid' should be 10–15. The most tasteful varieties show both a high sugar and a high acid content at picking time. The acid content decreases very fast after harvesting.

The flavour and the aroma of the fruit are determined by a specific volatile combination. In ripe fruits, there are hundreds of volatiles. In plums, there is a wide range in flavour from very poor to very rich. Taste and flavour mostly correlate with sugar content especially in European plums.

Some varieties have a touch of bitterness caused by polyphenoles often found in unripe fruits but sometimes also in ripe ones. Tannins impart a unique flavour preferred by some consumers (e.g., the variety 'German Prune'), but outstanding levels produce an undesirably bitter taste. In European plum, bitterness is well inherited by 'Čačanska najbolja'. As the taste of a fruit is determined by a complex of different traits, its inheritance is complicated. A crossing of varieties with poor taste may result in progenies with good taste. Good donors are 'Italian Prune, 'Hanita' (Fig. 10) and 'Harbella' especially for flavour and fine acid content. Generally, crossings between rich-flavoured plums give a high proportion of seedling with a rich flavour.

Varieties of *P. domestica* usually have more flavour than varieties of *P. salicina*. Therefore, a better eating quality is a breeding objective for Japanese plums in many countries. Meanwhile, there are some new varieties with better interior fruit quality.

Growers and breeders tried to improve certain characteristics of the European plum concerning fruit and tree characters over a period of more than 200 years. There is a demand to enlarge the amount of high-quality plum fruits used in human food. For optimal performance in the orchard, each genotype needs to be handled individually. It is very important to choose the right training system, to thin the fruits and to consider the individual demand of a variety on the location. Breeding for resistance to pest and diseases, especially for Sharka resistance, is one of the most important aims. The method of choice is the systematic hybridisation. Concerning some traits, the genetic gain obtained by the use of conventional hybridisation is well investigated. Good progress was achieved

Fig. 10 Interior fruit quality of European plum cultivars. (**a**) In prunes, flesh remains firm after baking. Varieties used for plum cake are not allowed to loose sap during baking. A, 'Hanka'; B, 'Katinka'. (**b**) 'Hanita', one of the tastiest European plum cultivars (*See* color insert)

Table 9 The genetic gain achieved for certain characteristics of European plum cultivars obtained by systematic hybridisation (according to Botu and Botu 2007).

Trait modified	Unit	Value of the trait in parents	Value of the trait in some selections	Genetic gain
Vigour of growth (T.C.S.A.)	%	100	90–100	±10
Fragility of branches ('Tuleu gras')	%	15–20	1–2	14–18
'Spur' type bearing	%	80–100	100	10–20
Blooming time (± days)	Days	0	±5	1–5
Fruit ripening	Days	0	–10;–20	10–20
Male sterility of female genitor	%	100	100	0
Fruit weight	g	25–35	40–95	15–60
Fruit size	mm	25–30	40–85	15–55
Stone adherence to flesh	%	7–10	5–7	2–3
Skin colour	%	100	100	0
Stone weight	g	1.0–3.0	1.5–4.0	0
Fruit taste	%	100	–5;–10	0
Dry matter	%	18–22	20–28	2–6
Yield	%	100	110–130	10–30
Resistance to Sharka disease	%	10–25	20–40	10–15

concerning the fruit size and the yield, as well as the enlargement of the fruit ripening time. The genetic gain depends on the crossing combination. Botu and Botu (2007) made an analysis of the genetic gain obtained in plum-breeding programmes in Romania (Table 9).

5 Resistance Breeding

5.1 Strategy

There are a lot of different kinds of damages in plum production caused by abitotic or biotic factors. In case there is any kind of variability in the gene pool of *P. domestica* and *P. salicina* concerning the reaction of the plants to the attack of a respective pathogen or to a abiotic environmental factor causing damages to the tree, the breeding of resistant or tolerant cultivars is, in principle, possible. If there is no variability in one of the two mentioned species, related species should be investigated. They can be used for interspecific hybridisation. Exemplarily, the breeding of plum cultivars resistant to Sharka disease will be described in details because Sharka is the most important disease in plums. The strategy for the breeding of cultivars resistant to other pathogens/abiotic environmental factors can be derived from these considerations.

There are several steps in breeding resistant cultivars: first of all, genetically fixed differences in the behaviour of single genotypes of the respective species against the pathogen must be detected. The more genotypes can be tested, the higher is the probability of finding resistance and/or tolerance. National gene banks can be used for obtaining a broad spectrum of different genotypes. For this kind of large-scale testing, a reliable resistance test has to be developed. Resistant genotypes must be selected in order to use them as a crossing partner. In advance or in parallel to a resistance breeding programme, the life cycle of the pathogen and the kind of reaction of the plant against it must be investigated. The durability of the resistance has to be estimated. For this purpose, a preferably large number of isolates of the respective pathogen must be used for inoculation tests. The mechanism of resistance or tolerance to the pathogen has to be described. By analysing progenies originating from different crossing combinations between resistant donors and other genotypes, the genetic determination of the resistance trait can be ascertained. If interspecific hybrids have to be used, methods for interspecific hybridisation have to be developed, for example, embryo rescue techniques. Before releasing a new variety, the respective genotype has to be tested under natural inoculation conditions on different sites for several years.

5.2 Sharka Resistance

The Sharka disease, for the first time described by Atanasoff (1933, 1935), is the most important disease in stone fruit production. It affects European (*P. domestica*) and Japanese (*P. salicina*) plum, peach (*P. persica*) including nectarine, apricots (*P. armeniaca*), sloe (*P. spinosa*), myrobalan (*P. cerasifera*) and, with minor impact, cherry (*P. avium* and *P. cerasus*). It is caused by a virus of the genus *Potyvirus*, the PPV. The most eye-catching symptoms are chlorotic rings and/or spots on the leaves of sensitive genotypes. Symptoms on the fruits are depressions on the surface and/or spots or rings which are especially well visible after removing the bloom of the fruits (Fig. 11). PPV-infected trees of

Fig. 11 Sharka symptoms on *P. domestica* cultivars (isolate of PPV-D strain). (**a**) 'German Prune': surface depressions on the fruit. (**b**) 'Habella': internal damages of the fruits. (**c**) 'German Prune': chlorosis on the leaf. (**d**) 'Katinka': chlorotic rings on the leaf (*See* color insert)

many cultivars show premature fruit drop. The fruit quality is low because of a high acid and low sugar content. The vegetative growth can be reduced. The lignification process is also influenced, which results in a poor elasticity of the shoots. Infected trees are detectable by the easier breaking of the infected shoots compared to healthy ones. This method can be used as a pre-test for Sharka infection even in leafless trees.

Multiple factors like biotic and abiotic environmental conditions, the degree of resistance or susceptibility, of tolerance or sensitivity of the respective cultivar, the virus strain or isolate and the age of the trees at the time of its infection with PPV influence the expression of PPV symptoms. If an old tree gets infected, the infection often remains limited to one or several branches of the tree, whereas the infection usually gets fully systemic if a young tree becomes infected. Sensitive genotypes can even die due to PPV infection. Yield losses, poor fruit quality and losses of trees are the most important economic impacts of PPV infections on stone fruit orchards. A detailed description of PPV symptomatology is given by Németh (1986).

There are two ways of avoiding economic damages caused by Sharka: (1) avoiding the infection of the trees with PPV and (2) using varieties showing only mild or no symptoms after PPV infection (tolerant and/or resistant varieties) (terminology used according to Cooper and Jones (1983)). The avoidance of infection could be carried out by the use of immune genotypes or by using cultivars and rootstocks that are resistant to the aphid vector of PPV. However, neither immunity nor vector resistance was found within European or Japanese plum (Rühl 1994; Grüntzig et al. 2001; Hartmann and Petruschke 2000; Hartmann and Neumüller 2006). Therefore, breeding programmes worldwide focus on gaining tolerant or resistant varieties. To a great extent, the degree of this tolerance or resistance depends on environmental factors and on the virus isolate infecting the plant. For several years, even widespread varieties, which were known not to show remarkable symptoms on fruits or to be quantitatively resistant, have suffered more and more from Sharka disease. Therefore, PPV causes increasing economic damage.

The terms ‘tolerance’ and ‘sensitivity’ describe the phenotypically visible reaction of the plant against infection with a pathogen. Tolerant genotypes show no or only mild symptoms. For fruit growers, it is most important that there are no symptoms on the fruits; therefore, they often prefer fruit-tolerant varieties that can show symptoms on the leaves but the fruits are only mildly affected by the pathogen (see Table 10). ‘Resistance’ and ‘susceptibility’ are corresponding terms describing the behaviour of the pathogen within the plant. In resistant cultivars, the virus concentration is lower than in susceptible ones and/or the systemic distribution of the virus within the plant is prohibited. To evaluate the resistance to PPV, the determination of the viral concentration (e.g., using semiquantitative ELISA or reverse-transcription-RT-PCR-techniques)

Table 10 Tolerance of some varieties of European plum against PPV according to the symptoms on leaves and fruits (Hamdorf and Hein 1989; Hartmann 1990; Rühl 1994; own experiments)

Variety	Leaves	Fruits	Variety	Leaves	Fruits
Anna Späth	–	o	Katinka	–	+
Auerbacher	–	–	Victoria	–	o
Bühler Frühzwetsche	o	+	Mirabelle de Nancy	+	+
Čačanska najbolja	+	+	Jalomita	–	–
Čačanska rodna	–	–	Ontariopflaume	+	+
Čačanska lepotica	o	+	Opal	+	+
Čačanska rana	–	+	Ortenauer	–	–
Carpatin	–	+	Oullins Reineclaude	+	+
Centenar	–	+	Pitestean		+
Chrudimer	+	+	Presenta	–	+
Czernowitzer	+	+	President	o	+
Elena	–	+	Ruth Gerstetter	–	+
Ersinger	–	+	Sanctus Hubertus	o	+
Fellenberg	–	–	Stanley	o	+
Felsina	–	–	Tegera	–	–
German Prune	–	–	Topend	+	–
Green Gage	–	o	Tophit	o	o
Harbella (Hoh 4515)	o	–	Topper	o	+
Haganta	o	o	Topfive	–	+
Hanita	–	+	Valjevka	o	+
Haroma (Hoh 4593)	o	+	Valor	+	–*
Herman	o	o	Zimmers Frühzwetsche	–	–

– sensitive (strong symptoms on the leaves/fruits)
o weakly sensitive/slightly tolerant
+ tolerant (very few symptoms on the leaves/fruits)
* During the 1980s, 'Valor' was considered to be fruit tolerant. During the last years, the variety suffers more and more from Sharka and shows symptoms on the fruits.

is necessary, whereas the tolerance can be estimated just looking at the phenotype of PPV-infected plants.

There are two kinds of resistances to PPV known in *P. domestica*: the so-called quantitative resistance and the resistance mediated by hypersensitive response. Quantitatively resistant cultivars have been known since a long time. The virus concentration in the leaves is diminished. However, they can get infected with PPV in the field by aphid transmission. The hypersensitivity resistance, which was discovered later, leads to a complete field resistance of the respective genotype: trees remain free from PPV in the orchard even under high infection pressure. Therefore, hypersensitive genotypes cannot be a source of inoculation of PPV in the field.

In order to test the Sharka resistance of *Prunus* species, the following parameters must be taken into account:

5.2.1 Virus Isolate

Until now, six different strains of PPV are known (PPV-D, PPV-M, PPV-Rec, PPV-W, PPV-EA, PPV-C) (James and Varga 2004; Myrta et al. 2006). Each strain itself consists of different PPV isolates. Each of these isolates can influence the viral concentration in the plum tissue and the development of symptoms in fruits and leaves of a given plum genotype. Thus, the choice of the PPV isolate for resistance tests can influence its result. Ideally, isolates that usually reach a high viral concentration should be preferred (e.g., the isolate 'CG' described by Kegler (1990)). In resistance tests, usually one isolate is used for all the seedlings. The most promising ones have to be tested with a broad range of isolates of each PPV strain in a second step.

5.2.2 Inoculation method

As the inoculation of woody plants using PPV-containing plant sap extract is very difficult and the results obtained by this method are not consistent, only the transmission using either natural vectors (aphids) or grafting is possible. The aphid transmission in the greenhouse mimics the natural transmission of PPV, but it is time- and labour-consuming and needs a lot of experience to get reliable results. The testing under natural inoculation conditions in the orchards as the only testing method is insufficient: it is well known that some individual trees of even highly susceptible varieties (e.g., 'German Prune' or 'Auerbacher') can remain free from PPV over a long period, whereas all the surrounding trees of the same cultivar get infected. Thus, the testing in orchards under high natural infection pressure cannot be used as test system for resistance screening purposes. Unfortunately, many investigations have used this method in the past (e.g., Minev and Dragoiski 1995). The results obtained by this method are more or less worthless, especially if conclusions concerning the choice of parents for resistance breeding are derived from those investigations (e.g., Lahmatova et al. 1998). There are different kinds of grafting suitable for inoculation. Often the chip budding method is used: chips of budwood cut from PPV-infected trees are budded into young plants of the genotype of interest. Depending on the number of chips, the PPV concentration within them and the size of the tree to be inoculated, the results obtained with the chip budding method can vary. Therefore, the grafting of budsticks of the genotype of interest onto heavily infected trees in the orchard, the grafting of them onto a virus-free myrobalan rootstock with PPV-infected interstem or the grafting on PPV-infected rootstocks in the greenhouse are the methods of choice. For testing hypersensitivity resistance (see below), these methods are necessary for phenotyping the response of a genotype to PPV infection and for determining the degree of hypersensitivity. The bigger the plant and the smaller the inoculum, the lower and slower is the reaction of the plant to the inoculation. For getting fast and clear results, the double-grafting method or the grafting on PPV-infected trees should be used as there is a continuous virus transport from the infected interstem or rootstock to the scion part.

5.2.3 Rating of Symptoms

The best time for the rating of the symptoms is in late spring time because during summer, the symptoms in the leaves may be masked. Viral concentration within the leaves may decline during high-temperature phases and therefore ELISA tests work best in late spring as well. In order to be able to compare the results of different experiments, some standard varieties have to be used in each resistance test: the PPV sensitive cultivars 'Italian Prune', 'Čačanska rodna' and 'German Prune', the tolerant cultivar 'Opal', the quantitatively resistant variety 'Čačanska najbolja' and the hypersensitive cultivar 'Jojo'.

5.2.4 Time of Inoculation

The time of inoculation during the phenological development of the plant has got high impact on the expression of symptoms. Inoculations by chip budding or aphids during summer or in the autumn usually provoke the development of symptoms not before the next spring.

5.2.5 Time of Observation

Using the double-grafting method, usually one growing season is sufficient for getting reliable results. However, in some genotypes, the Sharka virus remains latent for a few years after inoculation, especially when existing trees are inoculated in the orchards (Kegler 1990). Thus, at least those resistance screenings, which want to describe the viral impacts on the fruits, should be done over a period of at least 5 years. If the double-grafting method is used, usually one growing period is sufficient for getting reliable results.

5.2.6 Number of Tested Trees

The more trees are tested, the more meaningful are the results. In practical use, three plants per genotype in greenhouse tests and five plants per genotype in field tests are feasible. Depending on the biotic and abiotic environmental conditions, the reaction of the plant to PPV inoculation can vary. Therefore, the testing on several sites in different geographical regions is recommended.

Most of the reports about Sharka sensitivity or tolerance made in the last decades cannot fulfil all of the mentioned criteria. Most of them did not even determine the resistance of a genotype to PPV but its tolerance or sensitivity because only visible symptoms were rated. Therefore, it is difficult to draw any conclusions concerning the choice of parents for resistance breeding based on these investigations. Only few investigations have been carried out that produced reliable results due to the correct way of testing (e.g., Trifonov 1978; Sutic and Rankovic 1981; Kegler 1990; Petruschke and Schröder 1999). For the selection of parents for breeding, Kegler (1990) proposes to use three criteria:

a low expression of Sharka symptoms, a low virus concentration within the leaves and a low degree of systemic virus spread within the plant. Genotypes following these terms are donors of PPV resistance.

5.2.7 Inheritance of PPV Resistance

Most studies carried out during the last decades were dealing with the resistance or tolerance screening of existing cultivars. There are only very few systematic investigations concerning the inheritance of PPV resistance. In many cases, the term 'resistance' was uncorrectly used instead of 'tolerance'. Often, only some genotypes of high pomological value were tested instead of whole progenies of several crossing combinations so that no conclusions concerning the inheritance of PPV resistance or tolerance can be drawn. In those studies where whole crossing combinations have been screened, often inadequate test methods were applied. For example, Minev and Dragoiski (1995) planted seedlings in a field with heavy infection pressure by aphids and draw conclusions on the inheritance of PPV resistance. However, there was no artificial inoculation of the trees with PPV; therefore, one must doubt the conclusions drawn out of this experiment. Bivol et al. (1988) report about a multifactorial inheritance of the quantitative Sharka resistance. In most cases, the seedlings were, compared to the parents, intermediate concerning their degree of PPV resistance. Only the combination 'Graf Althans Reneclode' × 'Kirkes' resulted in a higher degree of PPV resistance in some single seedlings. In general, the progenies of the combination of two quantitatively resistant genotypes did not show higher degrees of resistance than the parents.

Until recently, it was assumed that breeding efforts could only result in tolerant and/or resistant varieties which only show mild PPV symptoms or have a lower virus titer within the plant tissue, but always get more or less systemically infected and are, therefore, a source of PPV for the further distribution of the Sharka disease (Atanasoff 1935; Lahmatova et al. 1998; Rankovic et al. 1995). However, Kegler et al. (2001) and Hartmann (2002) showed that in *P. domestica*, another type of resistance exists, which prevents the systemic infection of plum trees in the orchard by a resistance mechanism mediated by a hypersensitive response (hypersensitivity resistance). The trees of hypersensitive genotypes remain free from PPV in the orchard even if there is a high inoculation pressure by aphids. Thus, they are not a source of infection of neighbouring plants, a either in the nursery nor in the orchard. In the nursery, only trees free from PPV can develop so that the distribution of PPV over long distances can be avoided. Detailed studies on these resistance mechanisms have been carried out by Neumüller (2005). He described the response of more than 1150 genotypes of *P. domestica* originating from crossings between sensitive and hypersensitive genotypes at University of Hohenheim to artificial inoculation with PPV using the double-grafting method with a PPV-infected interstem described by Kegler et al. (1994).

The inheritance of the hypersensitivity was investigated. In this test system, hypersensitive genotypes show necrosis on the leaves and on the stems; the death of young shoot tips occurs as well. Among the descendants of all the crossing combinations tested, both sensitive and hypersensitive genotypes appeared as well as hybrids showing characteristics of both sensitivity and hypersensitivity (Neumüller et al. 2005; Neumüller et al. 2007). There is a smooth transition from sensitivity to hypersensitivity. From the phenotypical point of view, hypersensitivity is a quantitative trait (Neumüller and Hartmann 2008). In order to be able to describe the degree of hypersensitivity of an individual genotype, the ratings of the most important characteristics of hypersensitivity were used to describe the index of hypersensitivity (Table 11) (Neumüller and Hartmann 2008). Hybrids with a similar value of hypersensitivity index were grouped in four classes of hypersensitivity. Only members of two classes are of high pomological value.

Neumüller (2005) investigated the descendants of 26 crossing combinations originating from crossings with at least one hypersensitive parent of *P. domestica* (Table 12). The hypersensitivity fixed in the Hohenheim gene pool, originating from the crossing 'Ortenauer' × 'Stanley' (e.g., the variety 'Jojo') and effective to all PPV isolates tested up to now, showed a significantly better heredity than the one in descendants of 'K4'-hybrid, the hypersensitivity of which is specific to certain virus isolates (Fig. 12). Concerning the percentage of hypersensitive descendants, there were major differences in the combining ability of different genotypes. Unexpectedly, crossings between the hypersensitive variety 'Jojo' and varieties that are of high pomological value due to the excellent taste of their fruits, but highly PPV sensitive (like 'Fellenberg' or 'Felsina'), resulted in a high percentage of hypersensitive seedlings. As no maternal effects were observed, it was shown that the hypersensitivity resistance against PPV is encoded in the chromosomal DNA. It can be assumed that the hypersensitivity of European plum against PPV is controlled oligogenically.

Table 11 Hypersensitivity index (HI) and hypersensitivity classes (HC) (Neumüller 2005)

HI	HC	Description of HC, level of resistance of respective genotypes against PPV in the field
[0.00; 0.10[	0	Normally sensitive to PPV, genotypes can get infected in the field
[0.10; 0.40[	1	Slightly hypersensitive, genotypes can get infected in the field
[0.40; 0.70[	2	Normally hypersensitive, genotypes are usually completely resistant in the field
[0.70; 1.00]	3	Extremely hypersensitive, genotypes are completely resistant in the field (e.g., cultivar 'Jojo': HI = 0.71)

Table 12 Number of tested hybrids per crossing combination, percentage of genotypes belonging to different hypersensitivity classes (HC) and percentage of genotypes with typical Sharka symptoms on the leaves, ordered by the increasing percentage of hypersensitive genotypes in the breeding populations. The names of hypersensitive genotypes used as crossing partners are written in italics. (Neumüller 2005)

Crossing combination	Number*	HC 0 (%)	HC 1 (%)	HC 2 (%)	HC 3 (%)	HC 2+3 (%)†	PPV (%)‡	Ø HI§	Median (HI)‖
Čačanska lepotica × (*Ort*×*Stan 34*)	9	88.9	11.1	0.0	0.0	0.0	100.0	0.04	0.02
Jojo × *Clone 128*	43	72.1	16.3	7.0	4.6	11.6	95.4	0.12	0.00
Jojo × *Clone 108*	68	70.6	11.8	5.9	11.8	17.7	79.4	0.16	0.03
Jojo × Čačanska rodna	62	77.4	3.2	4.8	14.5	19.4	77.4	0.18	0.00
Jojo × Zwintschers Frühe	47	69.0	12.8	0.0	21.3	21.3	76.6	0.22	0.03
Čačanska najbolja × (*Ort*×*Stan34*)	14	71.4	7.1	0.0	21.4	21.4	85.7	0.20	0.00
Čačanska rodna × (*Ort*×*Stan34*)	30	66.7	10.0	0.0	23.3	23.3	86.7	0.21	0.02
Jojo × Hoh 1468	70	65.7	10.0	10.0	14.3	24.3	71.4	0.21	0.03
Jojo × Katinka	51	52.9	21.6	9.8	15.7	25.5	70.6	0.24	0.07
Jojo × Hauszwetsche, clone Schüfer	31	64.5	9.7	3.2	22.6	25.8	64.5	0.25	0.04
Jojo × Presenta	77	61.0	11.7	13.0	14.3	27.3	74.0	0.21	0.04
Elena × (*Ort*×*Stan34*)	63	58.7	1.6	9.5	30.2	39.7	63.5	0.33	0.03
Jojo × Hauszwetsche, clone Gunser	20	40.0	20.0	0.0	40.0	40.0	60.0	0.39	0.12
Jojo × Haganta	7	57.1	0.0	14.3	28.6	42.9	57.1	0.35	0.07
Hoh 4515 × *Jojo*	63	50.8	4.8	7.9	36.5	44.4	57.1	0.39	0.09
Fellenberg × *Jojo*	20	55.0	0.0	0.0	45.0	45.0	50.0	0.43	0.05
Jojo × Fellenberg	76	52.6	0.0	2.6	44.7	47.4	56.6	0.42	0.07
(*Ort*×*Stan34*) × Hanita	43	46.5	4.7	7.0	41.9	48.8	51.2	0.42	0.37
Hanita × *Jojo*	52	44.2	3.9	11.5	40.4	51.9	53.9	0.43	0.46
Jojo × Felsina	91	41.8	4.4	7.7	46.2	53.9	51.7	0.50	0.57

Table 12 (continued)

Crossing combination	Number*	HC 0 (%)	HC 1 (%)	HC 2 (%)	HC 3 (%)	HC 2+3 (%)[†]	PPV (%)[‡]	Ø HI[§]	Median (HI)[‖]
Jojo × (*Ort*×*Gerst17*)	14	42.9	0.0	0.0	57.1	57.1	50.0	0.51	0.76
Jojo × *Jojo*	33	42.4	0.0	3.0	54.6	57.6	42.4	0.47	0.72
Jojo × Hanita	49	40.8	0.0	18.4	40.8	59.2	51.0	0.47	0.56
(*Ort*×*Stan34*) × *Jojo*	47	34.0	2.1	14.9	48.9	63.8	36.2	0.54	0.69
Jojo × *Hoh 4465*	14	21.4	0.0	14.3	64.3	78.6	21.4	0.69	0.87
Hoh 4465 × *Jojo*	58	10.3	1.7	5.2	82.8	87.9	10.3	0.81	0.93
Total	1152	53.3	6.6	7.4	32.7	40.1	61.0	0.35	0.07

*Number of tested genotypes per crossing combination.
[†]Percentage of genotypes per breeding population belonging to HC 2 and 3 (i.e., high pomological value).
[‡]Percentage of genotypes with Sharka symptoms on the leaves.
[§]Arithmetic mean of the hypersensitivity index.
[‖]Median of the hypersensitivity index.

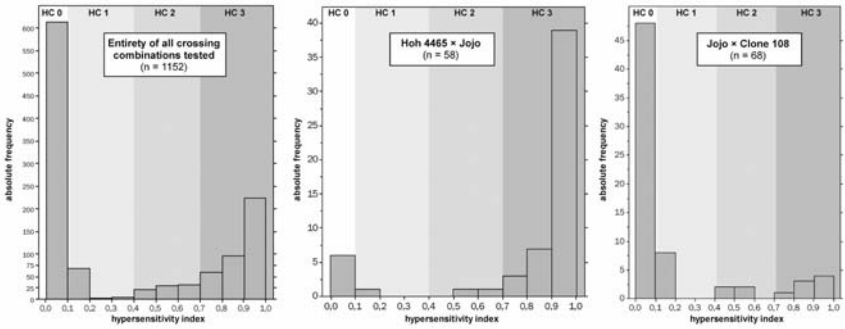

Fig. 12 Histograms of the hypersensitivity index of the entirety of all investigated genotypes and in two exemplary progenies. HC, hypersensitivity class. (Neumüller 2005)

The availability of hypersensitive genotypes provides, for the first time, the opportunity of reliably preventing the spread of Sharka virus into areas that have been free from PPV so far. For regions where PPV is prevalent, the cultivation of hypersensitive genotypes is the only possibility of not only minimising the economic damage caused by Sharka disease but also of avoiding it. The results presented concerning the heritability of the hypersensitivity show how to use this mechanism of resistance for breeding new cultivars efficiently. Presently, the breeding of varieties hypersensitive to PPV is the most promising approach for solving the problem of Sharka disease. In this respect, interspecific hybridisations for producing hypersensitive rootstocks have to be taken into account. Hypersensitivity might also be a promising tool for solving the Sharka problem of species related to the European plum like Japanese plum, peach and apricot.

5.3 Bacterial Cancer

The bacterial cancer (*Pseudomonas syringae* van Hall) is an important disease in most of the plum-producing countries. Plums on peach rootstock seem to be less susceptible than those on plum growing rootstock, and myrobalan seems to be less susceptible than 'Marianna' rootstocks (Ramming and Cociu 1991). Nothing is known about the inheritance of the resistance to bacterial cancer in plum. Independently of the genotype used as rootstock, the most important cultural practice for avoiding tree losses caused by *Pseudomonas* and other wound parasites is to avoid damages to the stem (Hinrichs-Berger 2004). It remains important for future breeding work to find sources of resistance against the bacterial cancer. In cherry, a resistance test was developed, which probably could be adapted to plum (Santi et al. 2004).

5.4 Brown Rot

The brown rot, caused by the fungi *Monilinia* spp., is one of the most important diseases of plums. It causes severe losses of the fruits especially in years with a lot of rain. Minoiu (1997) lists some varieties that are quite resistant to brown rot in Romania (e.g., 'Scoldus', 'Anna Späth', 'Prune d'Agen', 'Blue free', 'Bonne de Bry', 'Ruth Gerstetter', etc.) in contrast to some susceptible ones (e.g., 'Ontario', 'Kirke', 'Emma Leppermann', 'Early Laxton', etc.). However, systematical and comparative resistance screenings in plum are missing. Pascal et al. (1994) and Walter et al. (2004) present two inoculation methods for the screening of apricot, Japanese plum and peach to *Monilinia laxa*. They conclude that the resistance of the fruit flesh does not correlate with the resistance of the fruit skin (epidermis), but both parameters should be considered in resistance tests. One hybrid between *P. salicina* and *P. cerasifera* is described as quite resistant to inoculations into the flesh, whereas the investigated cultivars of *P. salicina* are more susceptible. Walter et al. (2004) describe methods for resistance tests in apricots, which could be used for resistance screenings in plums as well. However, a lot of impact factors such as local humidity, temperature and the strength of inoculum influence the screening results. Differences between the years are often larger than those between different cultivars. In general, varieties with high sugar content are more susceptible to *Monilinia* infections than the others, probably because of their higher tendency to cracking and better conditions for the fungal growth. No data are available on the genetic determination of brown rot resistance. The development of a reliable resistance screening method is outstanding.

6 Special Aspects of Rootstock Breeding

As it is usual in temperate fruit crops, both European and Japanese Plum are propagated vegetatively by grafting on rootstocks. The genotype of the roostock determines the later size of the whole tree; it affects the productivity and the fruit size. For plums, either seedling rootstocks (e.g. *P. cerasifera, P. domestica* 'Wangenheim') or clonally propagated rootstocks are used (e.g., selections of *P. insititia* and *P. cerasifera*, complex hybrids). Breeding efforts were reduced during the last decades worldwide. Meanwhile, rootstocks inducing medium size are available such as 'GF 655-2' (a selection of 'St. Julien A' (*P. domestica* spp. *insititia*)), 'Ishtara' (*P. salicina* 'Belsiana' × (*P. cerasifera* × *P. persica*)) or 'Jaspy' (*P. salicina* 'Methley' × *P. spinosa*). Many of the rootstocks used today in Europe result from the breeding work done in Bordeaux (France) initiated by Renaud (e.g., Renaud et al. 1991). It is now continued by Kleinhentz.

Only few varieties are successfully grown on their own roots such as 'German Prune' or 'Wangenheims'. However, other varieties are too vigorous and late bearing when not grafted on rootstocks, for example 'Bühler Frühzwetsche'.

In plum, PPV is not transmitted by seeds. Therefore, seed-grown rootstocks have an advantage over vegetatively propagated rootstocks. Myrobalan seedlings have been the most important rootstock in Western Europe and are still the most commonly used rootstock in Eastern and South Eastern Europe. However, most of the rootstocks propagated by seeds induce strong vigour. Thus, breeding efforts concentrate more and more on vegetatively propagated rootstocks, which induce small to medium-sized trees. Detailed descriptions of different rootstocks used for *P. domestica* are given by Wertheim (1998). Since that time, only few new rootstocks have been introduced.

6.1 Selection Procedure

Selection in rootstock breeding is much more difficult than the selection for fruit-bearing varieties because the effects of the rootstock can only be evaluated indirectly measuring parameters visible upon the scion, which is grafted on the rootstock. Only few parameters like the resistance against soil pathogens or the degree of virus resistance can be determined without using grafted trees. As the influence of the soil upon the performance of a scion–rootstock combination is high, different sites have to be used for the rootstock evaluation. There are some more factors, which have to be taken into account: the climate, the water supply, the availability of mineral nutrients, the training system, the soil cultivation and the height of the graft union above the ground. Depending on the strength of the vegetative vigour, which is induced by the rootstocks, their performance in different planting densities has to be tested. Using the same planting density for all the rootstock genotypes, important effects like intraspecific competition are neglected. As there are specific combining abilities between a respective rootstock and a respective scion cultivar, different rootstock–scion combinations have to be tested. Moreover, the trials have to be evaluated for at least 10 years, if possible much more, as delayed incompatibility may only occur in older trees. In most trials, the results obtained in the first years are over-estimated. Often, differences between the performance of the rootstocks decrease during a period of 10 years. It is important to use homogeneous plant material when starting a rootstock evaluation.

Very often, a two-phase selection is used in breeding programs: a scion cultivar is directly grafted upon all the potential rootstocks. Attention should be payed to use only budsticks free from virus and phytoplasma diseases. The performance of the trees is evaluated for a period of about 6 years. After that kind of pre-selection, the most promising rootstocks are propagated vegetatively either using suckers or, if suckers do not appear, cutting the trees just below the grafting union in order to induce the production of young shoots. The second selection is made on different orchard sites with at least three economically important varieties and with standard rootstocks as a comparison. This evaluation takes about 10–15 more years.

Depending on the breeding aim, a pre-selection of the seedlings might be appropriate. For instance, the nematode resistance or the hypersensitivity resistance against PPV can be checked before grafting scion cultivars upon the rootstocks. The pre-selected genotypes are clonally propagated (e.g., by hardwood cuttings), and the original seedlings are grown ungrafted. A number of clones can be grafted with different cultivars already in the primary selection. Using this kind of pre-selection, the number of genotypes to be evaluated can be reduced quite early in the breeding cycle.

When the seedlings are propagated vegetatively already in an early state, the rooting ability of a single genotype can be estimated, which is another criterion of high importance in pre-selection. However, one must take into account that juvenile seedlings usually can be very easily propagated vegetatively, whereas this might change after they have reached the adult phase. There are big differences in the rooting ability of hardwood cuttings as shown in Table 13. A hybrid between *P. domestica* and *P. spinosa* showed the highest percentage of rooted hardwood cuttings. Comparing *P. cerasifera* and *P. spinosa* as crossing partner of *P. domestica*, there are no differences in the rooting ability of the progenies (Table 14). Therefore, both the species seem to be equally suitable for interspecific hybridisation with European plum in rootstock breeding concerning the inheritance of easy vegetative propagation.

The most important breeding aims for rootstocks for European plum are easy vegetative propagation (hardwood or softwood cuttings, micropropagation in *vitro*), good compatibility with a broad range of varieties, cold hardiness, tolerance to calcareous soils, tolerance to wet and dry soil conditions, stability and durability of the grafting combination, resistance against important biotic environmental factors like parasites (e.g., nematodes such as *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *Pratylenchus vulnus* (Dirlewanger et al. 2004; Pinochet et al. 1996)), fungi and bacteria (e.g., *Pseudomonas* spp.),

Table 13 Percentage of rooted hardwood cuttings of different *Prunus* genotypes showing hypersensitive response after Sharka virus inoculation. Hardwood cuttings were treated with 0.5% (w/v) IBA and grown for 8 weeks at 4°C with bottom heat (15°C) at Technical University of Munich-Weihenstephan.

Parentage	Genotype	Percentage of rooted cuttings (%)
P. domestica × *P. domestica*	Hoh 4517	5
P. domestica × *P. domestica*	Hoh 4571	53
P. domestica × *P. domestica*	Jojo	21
P. domestica × *P. cerasifera*	Docera 5	17
P. domestica × *P. cerasifera*	Docera 6	48
P. domestica × *P. cerasifera*	Docera 9	58
P. domestica × *P. spinosa*	Dospina 20	38
P. domestica × *P. spinosa*	Dospina 22	76

Table 14 Rooting ability of softwood cuttings of seedlings of the crossing *P. domestica* × *P. spinosa* (average of 21 genotypes with 8 cuttings per genotype) and of seedlings of the crossing *P. domestica* × *P. cerasifera* (average of 12 different genotypes with 8 cuttings per genotype). Cuttings were made in August from seedlings sown in the same spring, treated with 0.5% of IBA immediately after cutting and cultivated for 6 weeks in a fog system

Cross-combination	Not rooted (%)	Weakly rooted (%)	Well rooted (%)
P. domestica × *P. spinosa*	41.7	7.1	51.2
P. domestica × *P. cerasifera*	41.7	0.0	58.3

viruses and phytoplasma, low tendency to suckering, strength of induced growth (development in the nursery and in the following years), tree habitus, regeneration ability and adaptation to different soil conditions with good ability for the uptake of mineral salts. The most important trait is the induction of high, early and regular yields, commonly expressed in the specific yield (kg/cm^2 stem cross-section area or kg/cm stem diameter). In European plum, a good regeneration ability of the tree is necessary to ensure regular yields with high fruit quality (especially good fruit size). Therefore, rootstocks that reduce the vegetative growth of the trees too much trees are not widely used as tend to age quickly coming along with a reduced fruit size.

The broad spectrum of plum rootstocks and their resistance to fungal diseases was summarised by Bernhard and Renaud (1990) and Wertheim (1998). Often, the origin of the rootstocks is not known definitely. Results concerning pathogen resistance vary from trial to trial. One of the most important factors influencing the test results are cultural practices such as pruning and fertilisation. The later the trees get nitrogen, the longer they will grow within the season. The later they start wood hardening, the more susceptible they will be to frost damage. Little damages to the stem are infection sites for pathogens such as *Pseudomonas*. However, detailed studies that compare the behaviour of a set of plum rootstocks against different pathogens are missing.

A lot of different *Prunus* species such as *P. cerasifera, P. domestica, P. spinosa, P. salicina, P. besseyi, P. tomentosa, P. pumila, P. americana, P. armeniaca, P. persica* and *P. dulcis* and its hybrids can be used as rootstocks for plums. 'Weito 6' and 'Weito 226' are selections of *P. tomentosa* made in Weihenstephan, Germany. These rootstocks are drought resistant and induce small, heavy cropping trees. However, they suffer in wet soils. Under suboptimal conditions, tree losses occur. Hybridisations of *P. tomentosa* and *P. domestica* are not successful. However, hybridisations of *P. tomentosa* with *P. besseyi* and other species are possible (Kask 1989). For instance, the hybrid 'VVA-1' (*P. tomentosa* × *P. cerasifera*) was introduced by Eremin as a dwarfing rootstock for plum and apricot as well as VVA-2, a seedling originating from open pollination of VVA-1, and VSV-1, a hybrid between *P. incisa* and *P. tomentosa* (Kask 1989). Only few is knows about the latter two rootstocks. The performance of 'VVA-1' was good in Dutch trials (dwarfing of scion growth, high cropping efficiency, good fruit

weight (Wertheim 1998; Peppelman et al. 2007)). There are casual reports on tree losses on wet soils. Further testing is needed. These examples show that interspecific hybrids can have great impact on the improvement of rootstocks for European and Japanese plum.

Recently, a plum rootstock breeding programme was started at Technical University of Munich-Weihenstephan. The aim is to develop semi-dwarfing and dwarfing rootstocks, which are hypersensitive to PPV (see below). Inter- and intraspecific hybridisations are carried out. If budsticks which are latently infected with PPV are grafted upon rootstocks showing a strong hypersensitive response against PPV, the budstick will either not grow or die after a short period of growth. In this way it is guaranteed that only trees free from PPV will leave the nursery. Therewith, the main way of distribution of PPV over long distances could be interrupted. Hypersensitive rootstocks could also be used for scions hypersensitive against PPV. Recent research is carried out on this subject.

The following method describes a new way of rootstock breeding developed at Technical University of Munich-Weihenstphan (Fig. 13). The seeds are sown in the year of the crossing (in August or September) and plants are cultivated to

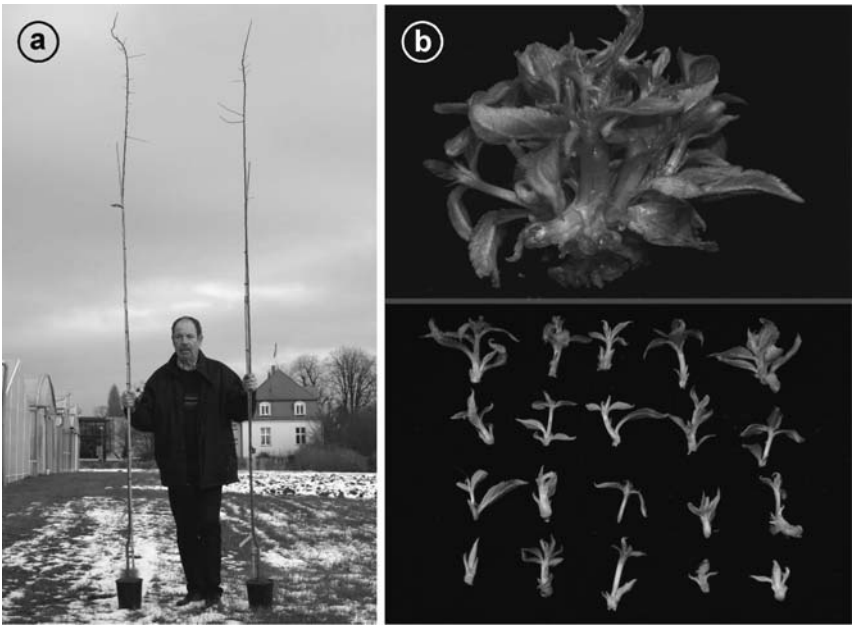

Fig. 13 Acceleration of rootstock breeding. (**a**) Rootstock propagation in the second year after pollination: interspecific hybrids (*P. domestica* × *P. spinosa*) grafted on myrobalan seedlings in March and grown in the greenhouse for one season. Height 3.9 m. Shoots are used as hardwood cuttings. (**b**) Propagation rate of a *P. domestica* rootstock clone in vitro. Multiplication rate is 1:20 within 6 weeks

a height of 1.5 m using artificial light until the late autumn. In November, some buds are taken from the stem of the plants and grafted on PPV-infected rootstocks, which had been in a dormancy period from August till October. During December and January, the inoculated plants are observed concerning their degree of resistance to PPV with special focus on hypersensitive genotypes. In this way, it is possible to describe the PPV resistance and the degree of hypersensitivity of each genotype not later than 10 months after pollination. From January to the beginning of March, the original seedlings undergo a dormancy phase at about 4°C. Only those seedlings being hypersensitive to PPV are kept. In March, the hypersensitive seedlings are grafted onto virus-free myrobalan rootstocks, about five plants per genotype, by chip budding. The seedlings are planted into the selection field. The grafted plants are cultivated in the greenhouse and can reach a height of 3–4 m until the autumn. Axillary branches are continuously removed. The terminal shoots are used for hardwood cuttings in the following winter. At least 10 rooted cuttings should result from each plant. They are immediately grafted with the varieties used for the evaluation of the rootstock characters of the seedlings and grown for a season in the greenhouse. Twenty-eight months after pollination, young trees (rootstocks grafted with test cultivars) are ready for planting in the orchard. For the most promising rootstock candidates, their ease-of-propagation *in vitro* is tested. Using this method, after only 2 years of pollination, the number of seedlings is reduced significantly and the testing of the suitability of the individual genotypes as rootstocks is started. In this way, the efficiency in rootstock breeding can be enhanced distinctly.

7 Breeding Activities

Similar to other fruit species, new plum cultivars were found for a long time as chance seedlings. The breeding of plums using scientific methods was started by Thomas Rivers (1798–1877), Ivan V. Mitschurin (1855–1935) and Luther Burbank (1849–1926).

7.1 European Plum

The first European plum cultivar obtained by controlled hybridisation is ‘The Czar’, developed by Thomas Rivers in England from a crossing of ‘Prince Engelbert’ and ‘Rivers Early Pacific’ in 1843 and introduced in 1873. The variety is named in honour of the visit of the Czar of Russia in the same year. ‘President’, a variety of commercial interest today, was found as chance seedling by Thomas Rivers. It was introduced in 1894. After the Second World War, plum breeding was intensified. A leading position in breeding held Romania

and former Yugoslavia. A sophisticated breeding programme for plums in a scientific level and with clear objectives was started in Romania in 1950 by Constantinescu and was continued by Cociu (Cociu et al. 1978). A short description of the Romanian varieties is given by Cociu et al. (1997). From 1967 to 1991, 26 new varieties were introduced in Romania. They originated from open pollination, simple crossing, double crossing, pyramidic crossing and also from the induction of mutagenesis using x-rays (Botu and Botu 2007). According to Burmistrov (1992), there are 10 breeding programmes in the former USSR. No information about these activities is available. Breeding in Yugoslavia at Čačak station started in 1959 and was done by Paunovic and later by Ogasanovic. Some very important varieties used today arose from this breeding programme, for example, 'Čačanska lepotica' and 'Čačanska rodna'.

During the last decades, breeding programmes were stopped in some countries because either the breeders retired or because of financial problems, for example, in England, France, Canada, Switzerland and Moldavia. In other countries, new plum breeding programmes were established like in the Czech Republic, Poland, Norway and some institutes in Italy.

A leading position in plum breeding of *P. domestica* holds Germany, especially in resistance breeding against PPV, using the hypersensitivity found in 'Jojo' and in a sister of this variety, the selection 'Ortenauer×Stanley 34' (Hartmann and Petruschke 2000). Plum breeding in Hohenheim was initiated in 1980 by Hartmann. From 1990 to 2006, 13 new varieties were released. Some of them are of commercial interest (Table 15). Important breeding aims are the combination of yield capacity and high fruit quality, the enlargement of the ripening time and the resistance breeding (Hartmann 1994). Resistance

Table 15 The commercially most important plum varieties in Germany and their origin. Sales volume (t/year) on the producer markets (ZMP 2005)

Variety	2000	2004	2000–2004	Origin of the respective variety
German Prune	4528	5159	4411	Unknown
Bühler	5035	2560	3508	Germany, 1854
Čačanska lepotica	3289	4561	2999	Yugoslavia (Čačak), 1961
Auerbacher	2103	3456	2407	Germany, 1875
Ortenauer	3257	1883	2341	Germany, 17th century
Hanita	957	2132	1460	Germany (Hohenheim), 1980
Čačanska rodna	873	1653	1165	Yugoslavia (Čačak), 1961
Ersinger	1522	984	1088	Germany, 1896
Top	483	1647	1009	Germany (Geisenheim), 1985
President	1298	801	826	England, 1894
Elena	349	1090	746	Germany (Hohenheim), 1980
Presenta	–	425	531*	Germany (Hohenheim), 1981
Herman	550	1031	517	Sweden (Balsgaard), 1952
Katinka	219	931	466	Germany (Hohenheim), 1982

* Average of the years 2004 and 2005

Fig. 14 New European plum cultivars. (**a**) 'Haroma', a Sharka-tolerant and tasty prune with high regular yield. (**b**) 'Jojo', the first completely Sharka-resistant variety (*See* color insert)

breeding is the main goal. Some of the new varieties are tolerant to PPV like 'Haroma' (Fig. 14a). The first absolutely Sharka-resistant European plum cultivar 'Jojo' was introduced in 1999 (Fig. 14b). Since 1996, hybrids between 'Jojo' and 18 different varieties and with a lot of other genotypes not yet released as varieties have been made (Hartmann 2007). About 2000 seedlings derived from 'Jojo' and its sister are waiting for selection.

A successful breeding programme was also set up by Jacob in Geisenheim since 1985 (Jacob 1998). From 1993 to 2005, 14 varieties have been introduced. Jacob grouped his varieties in five categories concerning their use: market varieties of medium size, very large-sized fruits, plums with a skin colour other than blue or violet, plums and prunes for distillery and big-sized mirabelles (Jacob 2002). With his retirement in 2005, the breeding activities were stopped. During the last years, no crossings have been made in Geisenheim just the selection using the existing plant material.

Within the framework of a new research project between the University of Hohenheim and the Technical University of Munich-Weihenstephan concerning the hypersensitivity of *P. domestica* and related species against the PPV, plum breeding was established in Weihenstephan by Neumüller in 2005. 4500 seedlings were obtained in the first four years.

In 1986, a breeding programme for European plum was started in Davis (California, USA). It is the only running breeding programme for this fruit species in North America. The fourth phase started in 2001 with the planting of 3800 seedlings (DeJong et al. 2002). A variety list of all European plums released between 1980 and 1990 is given by Bellini and Nencetti (1993). Running breeding activities for European plums are listened in Table 16.

7.2 Japanese Plum

For historical reasons, the breeding of *P. salicina* varieties has been concentrated to California and to the southern USA. California lists nearly 200

Table 16 Running European plum breeding programmes, objectives and released varieties

Country and breeding station	Start of breeding	Number of seedlings	Number of selections	Total number of released and name of the most interesting varieties		Main breeding objectives
Czech Republic (Holovousy)	1988	4500	12	3	Martina, Stana, Kompakta	Fruit quality, Sharka resistance
Germany						
– Geisenheim	1985	30000	150	14	Topfive, Topper, Tophit, Bellamira	Fruit size, yield, mirabelles with bigger fruits
– Hohenheim	1980	10000	268	12	Katinka, Hanita, Elena, Presenta, Jojo	Yield and fruit quality, ripening time, Sharka resistance, fruit size
– Weihenstephan (TUM)	2005	4500	5	–	–	Resistance, hypersensitivity to PPV in cultivars and rootstocks, fruit quality, ripening time, frresh market demands
Italy– Ancona	–	1000	15	–	–	Adaptation to the climate, productivity. Interspecific hybridisation
– Bologna	–	1500	117	2	Sugar Top, Prugna 29	Ripening period, fruit size and quality, ability for drying
– Firenze	–	1200	9	1	Firenze 90	Fruit size, ripening time
– Forli	–	5000	35	1	Liablue	Fruit quality, ripening time
Norway	1953	–	–	2	Edda	Larger fruits with good quality, high stable yield
(Njos)	1991	2000	15	–	–	
Poland (Skierniewice)	1991	5000	70	–	–	Sharka resistance, fruit size, quality, winter-hardiness, self-fertility
Romania (Bistritia, Pitesti, Vallea, Voinesti)	1950	65000	–	27	Pitestean, Centenar	Fruit quality, resistance to pest and diseases, ripening time, fruit size
Sweden (Balsgard)	1984	4400	–	7	Herman, Jubileum, Anita	Fruit size and quality, frost resistance
former Yugoslavia (Čačak)	1965	28000	239	12	Čačanska lepotica, Čačanska rodna, Valjevka	Sharka resistance, fruit quality, ripening time, storage, drying quality
USA (Davis/ California)	1986	15000	–	2	Tulare Giant, Sutter Prune	Prunes for drying, fruit quality, ripening time, resistance

Table 17 Major Japanese plum cultivars in California from 1975 to 1994 (Faust and Surányi 1999)

1975	%	1988	%	1994	%
Santa Rosa	20	Friar	18	Friar	20
Casselman	14	Red Beaut	12	Blackamber	12
Laroda	10	Santa Rosa	10	Angeleno	10
L. Santa Rosa	9	Blackamber	8	Santa Rosa	7
Red Beaut	6	Casselman	6	Red Beaut	7
El Dorado	6	Angeleno	5	Simka	5
Simka	5	Queen Ann	4	Laroda	4
Nubiana	5	Simka	5	Casselman	4
Royal Diamond	3	Black Beaut	5	Black Beaut	
Friar	3	El Dorado	3	Lesey	2

Japanese plum varieties in commercial production. But only varieties contribute to 75% of the whole production (Okie and Ramming 1999). The major plum cultivars in California are listed in Table 17. Active plum-breeding programmes are located in California, Georgia, Florida and Texas. In cooperation with the United States Department of Agriculture (USDA), the University of Davis started a breeding programme in 1932 and released some commercially important prunes for drying. Active plum breeding in California is made by USDA in Fresno. The breeding programme was established there by Weinberger in 1950s and is now under the supervision of Ramming. They released the following widespread varieties: 'Frontier', 'Friar' and 'Blackamber'. Currently, no crosses are made but more than 180 selections are tested.

In California, there are also some private breeders who have selected some important varieties such as the late-ripening 'Angeleno' (Table 18). During the last years, they introduced quite a lot of new varieties. Eighteen new Japanese plum varieties and several varieties from interspecific crossings have been released since 2003 (Ramming 2006). Private companies with large breeding programmes are, for instance, Zaiger Genetics, Bradford Farms and Sun World International. A high internal fruit quality, a high sugar content and a good balance of sugar and acid are becoming as important as the outer appearance of the fruits (Ramming 2006). The largest breeding programme outside of California is located at Byron in Georgia. From 1964 to 1995, more than 40,000 seedlings have been grown and eight varieties have been released (Okie and Ramming 1999). A comparison of the plum varieties bred at Byron with 'Santa Rosa' was made by Okie (2006). The breeding programme is continued by crossing Japanese plum cultivars with native plum species.

At University of Florida, a low-chill breeding programme has been developed (Sherman et al. 1992). With the release of 'Gulfbeauty' and 'Gulfblazze' in 1997, this work is phased out.

Table 18 Japanese plum-breeding programmes, objectives and released varieties

Country and breeder	Number of seedlings	Total number of released and names of the most interesting varieties		Main breeding objectives
USA				
– Bradford (Le Grand, California)	–	22	Red Beauty, Black Beauty, Grand Rosa	Ripening time, fruit quality, fruit size
– J. Gorbedian	–	8	Angeleno	Ripening time
– F. Zaiger (Modesta)	–	30	Golden Globe, Autumn Giant	Ripening time, fruit quality, fruit size
– Sun World Inc.	–	8	Black Diamand	Ripening time, improved flavour and size, colour
– Ramming, (USDA-ARS) (Parlier)	–	3	Black Spender	Eating quality, attractiveness, shipping and storage qualities
– B.D. Mowrey, D.W. Chain, T.A. Bacon (Waso)	–	8	–	Early- and late-ripening, fruit size, low chillin
– Okie, USDA-ARS (Byron, Gerogia)	50000	9	Black Ruby, Ruby Star	Disease resistance to *Xanthomonas* and *Pseudomonas*, leaf scald, late blooming
Brazil				
– Inst. Agron. de Campinas	–	9	–	Resistance to leaf rust, low chilling
– Inst. Agron. de Parana (Curbitiba)	30000		–	Resistance to leaf scald and bacterial spot
– EPAGRI Urussanga (Santa Caterina)	20000		–	Low chilling, diseases resistance to bacterial spot and leaf scald
South Africa (Infruitec)	20000	16	Pioneer, Sunkiss, Lady Red, Sungold	Large fruits, resistance to bacterial spot and bacterial canker, storage ability, low chilling, high productivity, full range of cultivars in ripening time and colour
Italy				
– Ancona	2000	–	–	Productivity, self-fertility, high and regular yield
– Bologna	1400	2	Black Sunrise, Black Glow	Ripening time, fertility, fruit quality
– Firenze	2300	2	–	Ripening time, regular yield, fruit quality
– Forli	2500	–	–	Quality, resistance to Phytoplasma diseases

In the Southern Hemisphere, there are some breeding programmes such as in Brazil, too. There are five Japanese breeding programmes in traditional plum-growing areas. Some varieties with low chill requirements have been released. Plum breeding in South Africa has been made at the Infruitec Center for Fruit Technology from 1972 to 1993. The aim has been to achieve large-fruited plums with resistance to diseases and good shipping quality. Eight varieties were introduced. One of them, 'Celebration', is a black-skinned variety. In Australia, plum breeding was established by Topp in 1967 at the Horticultural Research Station in Queensland.

Also in Japan, plum-breeding activities are mostly made by private breeders. In China, the main objectives in variety improvement are late ripening time, large fruit size, good cropping, eating quality, post-harvest behaviour and resistance to spot and other diseases (Weisheng 2007).

In Europe, breeding of Japanese plum is relatively new but will be important in the future because of the increasing demand for large-fruited plums. Some programmes were started in Italy.

8 Germplasm Conservation

Each successful breeding programme is based on the availability of a broad spectrum of potential parents. In order to breed new cultivars, the breeder must use the opportunities available in choosing the gene donors of all the diverse characters of the cultivars and species known all over the world. The International Board for Plant Genetic Resources has published a directory of germplasm collection (IBPGR 1989). The collection lists the breeder's working collection as well as national germplasm repositories. In 1995, 91 collections in 95 countries were assigned to *Prunus*.

In Europe, there is a *Prunus* cooperative programme for genetic resources with a network activity concerning the European *Prunus* database and the challenge for European collection. The European *Prunus* database (EPDB) was created under the auspices of International Plant Genetic Resources Institute (IPGRI) 20 years ago and was first maintained in Sweden. In the 1990s, it was transferred to Bordeaux (France) and developed to become an interactive database. The accessions incorporated mainly originated from Europe. The descriptions are made in consultation with the European *Prunus* Working Group. For plums, there are 15 specific descriptors of plant, fruit and agronomic characters. At present, more than 8000 accessions are registered and can be seen on the website of *Biodiversity International* (IPGRI). The main objective is to facilitate the long-term conservation of plant genetic resources and to encourage their utilisation. The participating gene banks are responsible to provide the name and identification of the accession, maintain the trees, provide the passport and characterisation data to the database manager and make scion wood available (Dosba and Zanetto 2005).

9 Recent Achievements and Prospects

The breeding of plum cultivars has to face two main problems: the Sharka disease and the fruit quality.

The availability of hypersensitive genotypes provides the opportunity of reliably preventing the spread of Sharka virus into areas that have been free from PPV so far. For regions where PPV is prevalent, the cultivation of hypersensitive genotypes is the only possibility of not only minimizing the economic damage caused by Sharka disease but also of avoiding it. Recent results concerning the heritability of the hypersensitivity show how to use this mechanism of resistance for breeding new cultivars efficiently. Presently, the breeding of varieties hypersensitive to PPV is the most promising approach for solving the problem of Sharka disease. In this respect, interspecific hybridisations for producing hypersensitive rootstocks have to be taken into account. Hypersensitivity might also be a promising tool for solving the Sharka problem of species related to the European plum like Japanese plum, peach and apricot.

Only high fruit quality can ensure the demand for European and Japanese plum fruits. Both taste and suitability for transport are key factors in future plum production. For European plum, large-scaled fruits with best internal quality have to be achieved. In the future, it will be necessary to develop varieties with excellent taste covering the whole range of the ripening time.

Except for Sharka, very few is known about the resistance of plum to the different pathogens, for example, *Monilinia* spp. In Japanese plum, resistance to European stone fruit yellowing (ESFY)—a phytoplasma disease—will become more important. Detailed studies are necessary in order to gather profound information on the interaction between plant and the parasite. Systematic breeding work and the development of effective resistance screening methods are necessary. Afterwards, the inheritance of the resistance traits can be studied. The role of molecular markers in the selection process plays, up to now, a minor role because they are not available. Before starting developing molecular markers, it is important to think about the expected costs and to weigh them against the costs of other selection procedures.

For the generation of genetically determined variability, the conventional breeding will remain the method of choice. In the past, mutation breeding and, other than expected, genetic engineering have failed to resolve problems in plum breeding. There is no indication that this could change during the next decade.

Interspecific hybridisation will gain special interest. It can be used for reducing the degree of ploidy in European plum, which is important for studying the inheritance of any trait of interest. Fruit quality and resistance traits can be transferred from European to Japanese plum and vice versa.

In rootstock breeding, interspecific hybridisation is commonly used. In the future, hypersensitive rootstocks might play an important role in resolving the Sharka problem in plum and related *Prunus* species. Recently, methods have been developed to fasten the breeding cycle in rootstock breeding considerably.

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Raspberry Breeding

Julie Graham and Nikki Jennings

1 World Production

Raspberries are grown in many parts of the world with production estimated at 482,763 MT (in 2005) (http:/ FAOSTAT.FAO.ORG). Europe is estimated to produce around half of all production (*Rubus idaeus* L.). This is an important high-value horticultural industry in many European countries, providing employment directly in agriculture, and indirectly in food processing and confectionary. Most raspberry production is concentrated in the northern and central European countries, although there is an increasing interest in growing cane fruits in southern Europe, for example, in Greece, Italy, Portugal and Spain. In many production areas, the fruit is grown for the fresh market, but in central Europe, for example, Poland, Hungary and Serbia, a high proportion of the crop is destined for processing. Major regions of production in North America include the Pacific North-West, California, Texas and Arkansas, as well as regions in New York, Michigan, Pennsylvania and Ohio. Chile, Argentina and Guatemala also have extensive production.

In Europe in particular, there has been increased interest in sales of raspberry fruits harvested from 'organic production'—farming based on methods relying entirely on crop rotation and avoidance of pesticide application (except certain substances currently permitted by the national regulatory authority for organic farming). However, with woody perennial crops, the difficulties of maintaining healthy productive plantations over many years are profound and it is too early to judge the overall success of these ventures in *Rubus* cane fruits. Increasing popularity of autumn-fruiting raspberries, in which late-season fruit is harvested from berries forming on the upper nodes of primocanes (Jennings and Brennan 2002), has extended the production season and the period of attack of some foliar and cane pests. Some very early spring fruits with high value can also be obtained from the remaining lower nodes of these over-wintered

J. Graham
Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK
e-mail: Julie.Graham@SCRI.AC.UK.

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primocane-fruiting types. Primocane-fruiting raspberries tend to be grown in the warmer areas of Europe where the temperature in autumn is relatively high and there is little risk of early autumn frosts. Interest has also been shown in extended-season production under glass or plastic structures in northern European countries, for example, Belgium (Meesters and Pitsioudis 1993; Verlinden 1995) and the UK (Barry 1995), and now in the Mediterranean fringe, for example, Spain and Greece, and this trend will affect their pest and disease status. To satisfy these production systems, long primocanes grown in northern regions, such as Scotland, are lifted, chilled and stored for long periods for planting in late spring for late summer harvest under plastic. The concept of extended-season production has been so successful that by careful manipulation of plant dormancy cycle and flower initiation, it is now possible to produce fresh raspberries in Europe for sale in almost all months.

Fruit has become important in the human diet due to increased consumers' awareness of healthy eating practices. In 2003, the global fresh fruit market was valued at £7.6 billion at current prices, having increased by just 3.9% since 1999. The fresh fruit sector accounts for 38.1% of the overall market and is gaining share due to continuing trend towards convenience food. Banana account the largest segment of the fruit sector with 22.5% of the market in 2003.

In terms of soft fruits, strawberries remain the best-selling soft fruit, but other fruits such as raspberry are gaining popularities because the increasing all-year round availability. Raspberries have always been attractive as fresh dessert fruits or for processing from frozen berries into conserves, purees and juices. It is interesting to see that raspberries were first used in Europe for medicinal purposes (Jennings 1988), but there is now heightened interest focused on these foods as major sources of antioxidants, such as anthocyanins, catechins, flavonols, flavones and ascorbic acid, compounds that protect against a wide variety of human diseases, particularly cardiovascular disease and epithelial (but not hormone-related) cancers (Deighton et al. 2000; Moyer et al. 2002). As a result, the consumption of these berries is expected to increase substantially in the near future as their value in the daily diet is publicised. A concerted effort by the public health authorities in Finland, for example, has promoted the consumption of small berry fruits to their populations (Puska et al. 1990) and in 2002, a similar initiative was launched in Scotland (Berry Scotland Project www.berry-scotland.com), though success here has yet to be demonstrated.

2 Raspberry Botany

Raspberries belong to the genus *Rubus*, one of the most diverse in the plant kingdom, comprised of a highly heterozygous series of some 500 species with ploidy levels ranging from diploid to dodecaploid (Jennings, 1988; Meng and Finn 2002). Members of the genus can be difficult to classify into distinct species for a number of reasons including hybridisation between species and apomixes

Fig. 1 (**A**) Red raspberry; (**B**) black raspberry; (**C**) protected crop of Glen Dell and (**D**) effect of root rot on field-grown plants (*See* color insert)

(Robertson 1974). These domesticated subgenera contain the raspberries, blackberries, arctic fruits and flowering raspberries, all of which have been utilised in breeding programmes. The most important raspberries are the European red raspberry (Fig. 1A), *R. idaeus* L. subsp. *idaeus*, the North American red raspberry *R. idaeus* subsp. strigosus Michx and the black raspberry (*R. occidentalis* L.) (Fig. 1B). *Rubus* subgenus *Idaeobatus* is distributed principally in Asia but also in East and South Africa, Europe and North America. In contrast, subgenus *Eubatus* is mainly distributed in South America, Europe and North America (Jennings 1988). The members of subgenus *Idaeobatus* spp. are distinguished by the ability of their mature fruits to separate from the receptacle. (For a list of *Rubus* species, subgenera and sections, see Skirvin et al. 2005.)

Details of the growth cycle of raspberry have been described by Jennings 1988). Raspberries are woody perennial plants with a biennial cane habit (Hudson 1959). Fruit is harvested annually from each plant, although both non-fruiting vegetative canes (primocanes) and fruiting canes (fructocanes) are present. This main season summer-fruiting crop is usually supported on a post-and-wire system designed to carry the weight of fruits and to protect canes from excessive damage due to wind, harvesting and cultivation. Primocanes are

produced in numbers excessive to requirements for cropping in the following season; so many must be removed by pruning in winter and early spring to reduce inter-cane competition and create an open crop canopy for efficient light capture. Old dead fruiting canes must also be removed by pruning after harvest. Such pruning operations remove sources of fungal inoculum from the plantation and are important for the long-term health of the crop.

Small (0.5–1.5 cm), white to pink flowers are initiated in the second year of planting. The gynoecium consists of 60–80 ovaries, each of which develops into a drupelet. There are 60–90 stamens. Raspberries produce copious amount of nectar and attract bees. The flowers of *Rubus* are structurally rather similar to those of strawberries, with five sepals, five petals, a very short hypanthium, many stamens and an apocarpous gynoecium of many carpels on a cone-like receptacle. Raspberries are an aggregate fruit, composed of individual drupelets, held together by almost invisible hairs. The one-seeded drupelets are set together on a small conical core (Jennings 1988). In *Rubus*, each carpel will develop into a small drupelet, with the mesocarp becoming fleshy and the endorcarp becoming hard and forming a tiny pit that encloses a single seed. Each drupelet usually has a single seed, though a few have two. Fruiting begins in the second year of planting, and in favourable conditions plantations can continue to fruit for more than 15 years. Fruit development occurs rapidly, taking only 30–36 days for most raspberry cultivars.

Primocanes and fruiting canes are in close proximity resulting in a complex plant architecture that provides spatial and temporal continuity for pests and pathogens to colonise a range of habitats (Willmer et al. 1986). The complex nature of the plant architecture also creates a barrier of foliage that impedes spray penetration of plant protectant chemicals, thus requiring specialised chemical application equipment (Gordon and Williamson 1988). Healthy plantations are expected to crop productively for more than 10 years, but this is only possible if the planting stocks and soils are free from persistent viral, bacterial and fungal diseases and certain pests, and hence the importance of quarantine arrangements and certification schemes to protect the propagation industry and fruit production (Jones 1991; Smith 2003).

Raspberry roots spread completely across the inter-row space, and these young canes ('suckers') developed from root buds (Hudson 1959; Knight and Keep 1960) must be removed, mechanically by tractor-mounted flailing equipment or by contact herbicides, to prevent competition of these suckers for light, water and nutrients with the crop.

3 Genetic Resources

Roach (1985) and Jennings (1988) gave accounts of the early domestication of red raspberry (*R. idaeus* L). During the 19th century, the North American red raspberry (*R. idaeus* subsp. strigosus Michx) was introduced in Europe and

subsequently crossed with the European sub-species (R. *idaeus* subsp. vulgatus Arrhen.). Five parent cultivars dominate the ancestry of red raspberry—'Lloyd George' and 'Pynes Royal' entirely derived from *R. idaeus* var. vulgatus and 'Preussen', 'Cuthbert' and 'Newburgh' derived from both the sub-species. Controlled crossing began slightly earlier in the USA than the UK with the introduction of Latham in 1914 (McNicol and Graham 1992). Domestication has resulted in a reduction of both morphological and genetic diversity in red raspberry (Graham et al. 1996; Haskell 1960; Jennings 1988) with modern cultivars being genetically similar (Dale et al. 1993; Graham and McNicol 1995). This restricted genetic diversity is of serious concern for future *Rubus* breeding, especially when seeking durable host resistance to intractable pests and diseases for which the repeated use of pesticides in some regions is ineffective, unsustainable or unacceptable for certain selected markets, such as 'organic production'. The gene base can and is being increased by the introduction of unselected raspberry clones and species material (Knight et al. 1989). The time required to produce finished cultivars from this material, however, can be considerable particularly if several generations of back-crossing are required to remove undesirable traits.

There are 30 *Rubus* breeding programmes in 19 countries, almost all of which are in Europe or North America. The Scottish bred cultivar 'Glen Ample', released in the mid-1990s, now dominates the UK market (www.fruitgateway.co.uk), superceding older varieties such as 'Malling Jewel', 'Glen Clova', 'Glen Prosen' and 'Glen Moy'. Glen Ample along with 'Tulameen' and recently the new cultivar 'Octavia' dominate the UK market and acreage due to their desirable fresh market characteristics. Serbia is a major world producer and exporter of raspberries, producing one quarter of the world tonnage. Ninety percent of the acreage is dominated by the North American cultivar 'Willamette'.

In recent years, consumer demand for fresh raspberries outwith the main production season has increased with high premiums being paid for fresh market raspberries. This demand is being met by imports from countries such as Spain, Portugal and Chile. Production of raspberries in either side of the main season is achieved through protected cropping under polytunnels (Fig. 1C), or glass, using novel systems of production to manipulate flowering and fruiting. Protected cropping and out of season production in European countries is expanding so that in areas of southern Spain, nearly 100% of fresh market raspberries are being grown under tunnels. The early season cultivar 'Glen Lyon' has a low chilling requirement, which makes it suitable for re-propagation and manipulation of canes and is currently the ideal variety for this production system.

These protected cropping systems have been adopted by the UK industry to improve fruit quality and extend the season. Since the majority of fresh market production goes to large supermarket chains, the demand for good fruit quality, flavour and shelf life is high. In other European countries, Pacific

Northwest-bred cultivars have led the industry, such as 'Meeker', 'Willamette' and 'Tulameen'. Primocane-fruiting cultivar 'Heritage' has led the industry in many countries. In Scandanavia, the hardy Norwegian variety 'Veten' has been the mainstay for many years; now 'Glen Ample' has taken the lead.

In the USA, 'Meeker' and 'Willamette' developed in the mid-1900s are the primary cultivars, although recent publicly developed cultivars 'Cowichan' and 'Coho' are being widely planted. Black raspberry (*R. occidentalis* L.) production has traditionally been concentrated almost completely in Oregon, 'Munger' and 'Jewel' being the leading varieties; however, a strong South Korean industry has developed over the past 5 years.

4 Breeding Principals and Objectives

Rubus breeding is hampered by several genetic problems including polyploidy, apomixes, pollen incompatibility and poor seedling germination. The highly heterozygous nature of the germplasm requires evaluation of large seedling populations. Breeding is based on a generation-by-generation improvement in breeding stock through selection and intermating individuals showing promise of producing superior progeny. This average improvement in the progeny of breeding stock resulting from intermating selected parents is called response to selection. (For a review, see Hansche 1983).

A survey in 2000 indicated that there are around 30 *Rubus* breeding programmes in 19 different countries, mainly in Europe and North America. Breeding programmes sponsored by end-users or government-funded programmes aim to develop appropriate germplasm enabling their particular industry to realize its potential, and thus goals vary from programme to programme. Many are faced with the same challenges, however, as the industry requires cultivars with excellent quality, higher yield, greater pathogen resistance and adaptation. As new problems arise and new production systems are developed, breeding programmes are faced with meeting these demands with new cultivars. The core primary objectives in raspberry-breeding programmes include high-quality fruit, good yield, shelf life and suitability for shipping, if for the fresh market, suitability for mechanical harvesting for the processing market, adaptation to the local environment and improved pathogen resistance. Future changes in environmental, cultural and agronomic practices within the industry will impact strongly on both the nature of the germplasm required for the future and also the likely pest and disease problems.

Fruit quality must always be the major objective of any fruit-breeding programme. While many characteristics are important in the successful acceptance of new cultivars, fruit quality must be considered the premier factor. Flavour, appearance and shelf life are the main attributes of fresh market quality. Flavour is a highly subjective trait but can be broken down into

multiple descriptors for taste, texture and other sensory characteristics. Good, acceptable raspberry flavour tends to be fruity, sweet and floral with some acidity and no bitterness (Harrison et al. 1999). Colour, brightness, size and shape contribute to appearance. A naturally dark colour can be perceived as overripe by fresh market retailers, whereas a darker colour is desirable for processing. Large size is an attractive characteristic to both consumers and producers as it is more cost-effective to pick.

The biennial cropping habit of raspberry means that both fruiting and vegetative canes exist together. Plant habit is important in plantation management and has a major effect on yield potential. In summer-fruiting types, the most important plant characteristics include number and height of young cane, consistency of bud break, internode length and lateral length and position. In primocane-fruiting types, the amount of branching and extent of lateral development on the primocanes are major yield components. In both types erect, spineless canes are desirable.

Machine harvesting for processing raspberries is the standard practice for most major raspberry production regions around the world and is essential where picking labour is expensive or unavailable. Despite advances in machine technology, it appears that the major improvements in harvesting will come from plant breeding (Cormack 1989). No single attribute has been found to determine successful machine harvest –ability, but a range of interacting traits governs harvest performance. A suitable genotype must have uniform strong vigour and good cane density with an upright habit. Medium-length laterals with good fruit presentation are also desirable. Maturity, physical shape of the berry and receptacle all contribute to ease of pick. This will help ensure that a high percentage of uniform, ripe fruit with acceptable process quality and minimal green fruit are harvested throughout the season (Hall et al. 2002).

In the UK and Europe, a transformation in cultivation practices has occurred from outside-field plantations to protected cropping systems. Such changes in agronomic practices affect plant growth, seasonality and fruit quality and have implications for a shift in pest and pathogen pressures. For example, in Scotland, pests such as two-spot spider mite (*Tetranychus urticae*) and diseases such as powdery mildew (*Sphaerotheca macularis*) are not a problem in field plantations but occur readily in tunnel production systems. Recently, breeding programmes in the UK have responded to this change in production by trialling and selecting germplasm under protected cropping systems. This will help to identify suitably adapted germplasm for commercial trialling and eliminate the most susceptible seedlings early in the breeding process.

While fruit quality must remain the priority in any commercial breeding programme, the incorporation of novel resistance/tolerance to pests and diseases is regarded as essential for the development of cultivars suitable for culture under integrated pest management (IPM) systems. Sources of resistance in diverse *Rubus* spp. to many pests and diseases have been identified and

exploited in conventional cross-breeding (Keep et al. 1977; Jones et al. 1984; Jennings 1988; Knight 1991; Williamson and Jennings 1992). However, germplasm bearing single-resistance genes, when planted over extensive areas, can eventually be overcome by the rapid evolution of new biotypes of pests so that new types of host resistance are required to sustain plant protection (Birch et al. 2002; Jones et al. 2002).

Pest and diseases of raspberry in Europe have been extensively reviewed in Gordon et al. (2006). Major pest and diseases will be briefly discussed. Root rot, cane diseases and raspberry bushy dwarf virus (RBDV) are problems worldwide with aphids, cane midge and beetles being serious problems in Europe.

Root rot diseases have always been a problem in North America but were not regarded as a problem in Europe until the 1980s when *Phytophthora* root rot emerged as a major problem of raspberry with outbreaks in the UK (Duncan et al. 1987), Scandinavia and Germany (Seemüller et al. 1986) (Fig. 1D). Raspberry root rot became a serious problem throughout temperate Australia during the unusually wet years of 1994–1996 with *Phytophthora fragariae* var. *rubi* (Wilcox et al. 1993) identified as the major causal agent. This disease is now the most destructive disease of raspberries. Affected canes die in the first year of growth or their buds fail to emerge at the start of the second growing season. Alternatively, emerged laterals wilt and die at any time from emergence until late in fruiting. The almost simultaneous outbreaks of a new disease across Europe in traditional raspberry-growing areas (e.g., raspberries have been grown in Tayside, Scotland for more than century) suggested that the disease had spread through the propagation network and had been distributed to farms in new planting materials. Introduction of new and highly susceptible cultivars sought vigorously by industry was a major factor in disease spread.

The prevention of new outbreaks must become the underpinning philosophy in control strategies for root rot. Ensuring that the planting material is free of disease is most effective strategy. The pathogen is unlikely to be present widely in soil where raspberries have never been grown previously. Screening cultivars of red and other raspberries and wild *Rubus* species have identified potential sources of resistance. 'Latham' and 'Winkler's Sämling' were identified early as having significant resistance. Species material, such as *R. strigosus* and *R. ursinus*, have also been identified. Genetic resistance through plant breeding offers a feasible and effective method of control, but because of the time involved in combining resistance with other desirable traits, e.g., fruit size and quality, it has not yet had the anticipated impact in commercial production. More research to find resistance genes and breeding is required, especially marker-assisted breeding. Future breeding plans with respect to root rot resistance are underpinned by attempts to develop molecular markers linked to resistance to improve and accelerate selection efficiencies (Graham and Smith 2002). It seems likely that this potent disease will be managed most effectively in the future by enhanced host resistance.

Botrytis cinerea accounts for severe losses in yield in most seasons and can cause devastating losses post-harvest if control measures are inadequate, especially in regions with moderate rainfall during blossom and harvest. This pathogen is difficult to control because there are multiple infection sites and no strongly resistant cultivars available to growers. Unfortunately, conventional breeding has yet to produce cultivars that are highly resistant to grey mould, although there are some cultivars and selections with high levels of resistance to cane botrytis (Williamson and Jennings 1992). However, considerable variation in resistance to botrytis fruit rot has been reported in *R. crataegifolius* and *R. occidentalis*. Resistance to cane botrytis has recently been mapped and association with gene *H* conferring cane pubescence confirmed (Graham et al. 2006).

RBDV is the most common virus of *Rubus* worldwide (Martin 2002). This is of greatest concern in North America, particularly in the Pacific Northwest where the virus has reached epidemic proportions, probably due to the planting of newer cultivars that lack resistance. RBDV is a pollen-borne virus, which may cause a crumbly fruit symptom with foliar symptoms ranging from none to a bright yellow chlorosis, and in a few cultivars, there is a stunting of the plants. Not all infected plants show crumbly fruit. In black raspberry, RBDV does not cause significant fruit losses or symptoms but can reduce cane number and vigour. Since the virus cannot be controlled with chemical applications, the best means of controlling RBDV is with the use of immune cultivars; however, the occurrence of resistance-breaking isolates (RB) poses serious risks for future control.

The large raspberry aphid (*Amphorophora idaei*) is the most important aphid species found on raspberry in northern Europe causing direct feeding damage to susceptible cultivars (Fig. 2), but its major importance is as a vector of raspberry viruses that cause serious decreases in plant vigour (Jones 1986; Birch and Jones 1988). Raspberry cultivars containing genes for resistance to the large raspberry aphid have been in commercial use in the UK for more than

Fig. 2 Large raspberry aphid (*See* color insert)

40 years (Briggs 1965). The resistance largely depends on two single major genes that control aphid numbers and subsequently the spread of the viruses they transmit (Jones 1986). Unfortunately, virulent biotypes of *A. idaei* that can break these specific resistance genes in their raspberry host plants have now developed (Birch et al.1994; Birch et al. 2002).

The raspberry beetle (*Byturus tomentosus* De Geer) is a major pest of cultivated raspberry and hybrid berries in many countries of Europe and frequently found in fruits of wild raspberries and blackberries. Adult beetles can damage the buds and flowers by feeding on them in the spring and early summer, but the most important damage in Europe is caused by larvae. They browse on the surface of drupelets on the developing fruit resulting in discoloured or contaminated ripe fruit leading to rejection or down-grading of the crop. This damaged fruit can become infected by botrytis (*B. cinerea*), thus further reducing the storage of the ripe fruit (Woodford et al. 2002). Wild *Rubus* species, including *R. coreanus*, *R. craetigifolius, R. occidentalis* and *R. phoenicolaesius*, have been used as sources of resistance to raspberry beetle (Briggs et al. 1982) in breeding programmes. As yet, no commercial cultivars are available. Little is known about the mechanism(s) of resistance to raspberry beetle involved in wild *Rubus* species or crosses derived from them.

Feeding damage caused by raspberry cane midge (*Resseliella theobaldi*) larvae predisposes raspberry canes to the disease known as 'midge blight', which is responsible for major losses in raspberry in many parts of Europe (Woodford and Gordon 1978). In most of Europe, midge larvae only colonise splits in the bark of primocanes, but in Scandinavia, larvae have been reported from splits in fruiting canes of the cultivars 'Veten' (Sorum and Stenseth. 1988) and 'Ottawa' (Dalman 1991). The raspberry cv. Glen Prosen and the hybrid berries Tayberry and Loganberry do not readily split their rind in the spring, and are rarely affected by 'midge blight' because female midges are unable to find suitable oviposition sites unless they are caused by mechanical means. Other *Rubus* species and crosses have been investigated as sources of resistance to raspberry cane midge. *R. parviflorus, R. odoratus* and F_2 crosses of *R. crataegifolius* × *R. idaeus* were found to be resistant when exposed to raspberry cane midges.

5 Breeding Techniques

Breeding in raspberry is carried out by hybridisations between cultivars and/or species with desirable characteristics for multiple generations. Each cycle of crossing involves a cycle of greenhouse screening and field observation. Crosses tend to be done outwith the normal flowering period requiring dormant plants to be dug in autumn and placed in cold storage for around 6–10 weeks. The plants are then moved into an insect-proof greenhouse where the temperature is

raised gradually from 10°C to 20°C over a 3-week period. Day length is set at 16 h. Plants break bud, produce laterals and begin to flower approximately 4 weeks later. Open flowers are collected into a Petri dish for use as a pollen source, dried at room temperature and stored with a desiccant at 4°C. Closed flower buds are emasculated with a scalpel and are ready to pollinate once the stigma have become receptive (approx. 48 h after emasculation). The pistil is pollinated with an artist's paintbrush. All tools and hands are sterilised with absolute alcohol between crosses, and all excess flower buds are removed to minimise pollen transfer in the greenhouse environment; therefore pollen bags are not required. Parent plants are sprayed for pests and diseases as appropriate for the duration of crossing.

Fruit from each family is collected when ripe and left in a pectinase solution overnight at room temperature. The pulp is separated from the seed by blending the mixture for 10 s in a domestic blender. The mixture is left to settle for 1 min; viable seed will sink to the bottom and pulp and non-viable seed will float to the top. The pulp is decanted from the viable seed. The seed is rinsed by filling the jug with tap water, leaving to settle and decanted. The rinse cycle is repeated three times, until the tap water is clear. The seed, which is clean and free of any pulp, is left to dry overnight on filter paper. Dry seed are stored in glassine bags (100 × 70 mm) with a desicant at 4°C.

Up to 1000 seed/family are scarified in acid, assuming 15–20% germination. Remaining seeds are stored in case of poor germination. Seed must be clean and dry before scarification in acid. Seed is transferred to a boiling tube (~500 seed/tube) with concentrated sulphuric acid for exactly 20 min and rinsed by pouring the seed and acid through a metal sieve, secured by a retort stand, and rinsing with tap water for 10 min. Seed should be submerged under the water during this period. Seed is then submerged in calcium hypochlorite solution for 6–10 days with stirring every day, and the solution should be changed once during this period. Once the seed coat has been scarified with acid, it is important that the seed is not left to dry out. Seed is rinsed under tap water for 10 min and mixed with damp vermiculite. The mixture is stored in a sealable bag at 4°C for 6 weeks. After this period, the seed and vermiculite is treated with GA_3 (3 ppm) and left at room temperature overnight.

The seed and vermiculite is sown onto Bulrush Brown/Black peat in a seed tray and covered with a fine layer of dry vermiculite. The trays are incubated at 20°C. Seeds should begin to germinate within 7 days.

6 Molecular Markers in Breeding

Breeding methods used in raspberry have changed very little over the last 40 years or so. Little novel germplasm has made its way into commercial cultivars. However, with the narrowing genetic base coupled with the increasing

demands from consumers, new breeding methods are required to meet demands. The speed and precision of breeding can be improved by the deployment of molecular tools for germplasm assessment and the development of genetic linkage maps. The development and application of molecular markers have been reviewed by Antonius-Klemola (1999), Hokanson (2001) and Skirvin et al. (2005). The development of SSR markers (Graham et al. 2002; Stafne et al. 2005) has allowed the development of a raspberry genetic linkage map. This facilitates the development of diagnostic markers for polygenic traits and the identification of genes controlling complex phenotypes. Understanding the genetic control of commercially and nutritionally important traits and the linkage of these characteristics to molecular markers on chromosomes is the future of plant breeding. Red raspberry (*R. idaeus*) is a good species for the application of such techniques, being diploid ($2n = 2x = 14$) with a very small genome (275 Mbp). Indeed, the haploid genome size of raspberry is only twice the size of *Arabidopsis*, making it highly amenable to complete physical map construction, thereby providing a platform for map-based gene cloning and comparative mapping with other members of the Rosaceae (Dirlewanger et al. 2004). The availability of abundant genetic variation in natural and experimental populations and adaptation to a range of diverse habitats (Graham et al. 1997; Marshall et al. 2001; Graham et al. 2003) offers researchers a rich source of variation in morphology, anatomy, physiology, phenology and response to a range of biotic and abiotic stress. The ability to vegetatively propagated individual plants provides opportunities to capture genetic variation over generations and replicate individual genotypes to partition and quantify environmental and genetic components of variation of genetic linkage maps. These are necessary to develop diagnostic markers for polygenic traits and, in the future, possibly identify the genes behind the traits. The first genetic linkage of raspberry has recently been constructed (Graham et al. 2004). This 789 cM genetic linkage map was constructed utilising a cross between the phenotypically diverse European red raspberry cultivar Glen Moy and the North American cultivar Latham. SSR markers were developed from both genomic and cDNA libraries from Glen Moy. These SSRs, together with AFLP markers, were utilised to create a linkage map. An enhanced map with further SSR and EST-SSR and gene markers has recently been completed (Graham et al. 2006). This work has highlighted the importance of maps and markers with demonstration of the tight association between gene *H* and resistance to cane botrytis and spur blight (Graham et al. 2006).

7 Problems and Unknowns

For some breeding objectives, a lack of appropriate germplasm may seriously hamper progress. In these cases, genetic manipulation may be the way forward (Graham et al. 1996). This may not be possible in Europe in the near future,

however, due to strong opposition from certain groups and sectors. Genetic manipulation in *Rubus* has been reviewed by Skirvin et al. (2005).

Most commercial raspberries are now maintained, propagated and sold as disease-indexed plants using micropropagation. Estimates of somaclonal variation of 1–3% per generation may be conservative with other estimates of 10% (Larkin et al. 1989).

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Breeding Walnuts (*Juglans Regia*)

Gale McGranahan and Chuck Leslie

1 Introduction

1.1 Origin and History

Ancestral forms of walnut once spanned Europe, Asia, and the Americas as far north as Alaska. Climate changes altered the geographic distribution and further evolutionary pressures resulted in the 21 species of *Juglans* in existence today. All species produce nuts, but the Persian or English walnut (*Juglans regia*) is the only species widely cultivated for nut production and will be the focus of this chapter. Other species are grown for timber (e.g., *J. nigra*, eastern black walnut) or are used as rootstocks for Persian walnut (e.g., *J. hindsii*, northern California black walnut).

Persian walnuts are native to the mountain ranges of Central Asia extending from Xinjiang province of western China, parts of Kazakhstan, Uzbekistan, and southern Kyrgyzstan, and from the mountains of Nepal, Tibet, northern India, and Pakistan west through Afghanistan, Turkmenia, and Iran to portions of Azerbaijan, Armenia, Georgia, and eastern Turkey. Small remnant populations of *J. regia* may have survived the last glacial period in southeastern Europe, but the bulk of the wild *J. regia* germplasm in the Balkan Peninsula and much of Turkey was most likely introduced from Iran and eastern Turkey by Greek commerce and settlement several thousand years ago (Zohary and Hopf, 1993). From Greece, the cultivation spread to Rome, where walnuts were known as *Jovis Glans*, or Jupiter's acorn, from which comes the genus name *Juglans*. From Italy, *J. regia* spread to what are now France, Spain, Portugal, and southern Germany (Leslie and McGranahan, 1998). The word walnut may be derived from "wealh nut," "wealh" meaning foreign in Anglo-Saxon or old German. Trees of this species were in England by 1562, and nuts were brought

G. McGranahan
Department of Plant Sciences, Mail stop 2, One Shields Avenue, University of California, Davis, CA 95616, USA
e-mail: ghmcgranahan@ucdavis.edu

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to America by the earliest settlers. The American colonists are said to have called the species "English" walnut to distinguish it from the native American eastern black walnut (*J. nigra*). *J. regia* germplasm in China is thought to have been introduced from central Asia about 2000 years ago and in some areas became naturalized, although there appear to be natural stands in the Xinjiang Uygur Autonomous Region of China.

1.2 Production

Persian walnuts are grown in North, Central, and South America, Europe, Asia, and the former Soviet Republics, and to a limited extent in Oceania and North Africa. Over 1.4 million metric tons were produced in 2003 (FAOSTAT data, 2004). China leads world production, followed by the USA, Iran, Turkey, Ukraine, Romania, France, and India (FAOSTAT data, 2004). The major exporters are the USA, which exports 115,000 MT, followed by France (23,000 MT), China (22,000 MT), and India (17,000 MT). Shelled walnuts make up 62% of the exports. Several of the major producers consume the bulk of their walnut production domestically, for example, China, Iran, and Turkey. Chile, on the other hand, exported 13,000 MT in 2003, almost its entire production. China has encouraged its growers to plant high-value crops like walnuts and expects to have over 1 million hectares of walnuts by 2012. New areas of production are also developing in Chile and Argentina.

1.3 Uses and Nutritional Composition

Walnuts have had many uses over time. Although now the dried walnuts are consumed either as a snack or dessert nut or in baked goods, in times past they had a variety of uses. They were thrown by the grooms in Roman weddings to signify maturity. In the middle ages, they were used to ward off lightening, fevers, witchcraft, and epileptic fits. According to the Doctrine of Signatures (16–17th centuries), tinctures of the husk were used for ailments of the scalp and the kernel could be used to sooth the brain (Rosengarten, 1984). Currently, recipes can be found for green walnut pickles and walnut liqueurs, and in parts of the world, the undried walnuts, 'fresh walnuts', are eaten after peeling off the bitter seed coat.

Oils are the most prominent nutrient in walnuts (Tables 1 and 2). Recently, the health benefits of the oils, especially the omega-3 fatty acid, in walnuts have been investigated and found to be highly beneficial. In one study that compared a low fat and modified low diet, it was shown that including 8–10 walnuts per day improved the high-density lipoprotein (HDL) to total cholesterol in men and women diagnosed with type 2 diabetes. The low-density lipoprotein (LDL) was also decreased by 10% (Tapsell et al., 2004). In another study (Ros et al. 2004), a Mediterranean diet was compared to a similar diet in which

Table 1 Nutrient composition of walnuts

Nutrients		Amount in 100 g of kernel
Proximate	Water	4.07 g
	Food energy	654 kcal
	Protein	15.23 g
	Total lipid	65.21 g
	Carbohydrate	13.71 g
	Dietary fiber	6.7 g
	Ash	1.78 g
Minerals	Calcium	98 mg
	Copper	1.59 mg
	Iron	2.91 mg
	Magnesium	158 mg
	Manganese	3.41 mg
	Phosphorus	346 mg
	Potassium	441 mg
	Selenium	4.9 μg
	Sodium	2 mg
	Zinc	3.09 mg
Vitamins	Ascorbic acid	1.3 mg
	Thiamin	0.34 mg
	Riboflavin	0.15 mg
	Niacin	1.13 mg
	Pantothenic acid	0.57 mg
	Vitamin B6	0.54 mg
	Folate	98 μg
	Vitamin A	20 IU
	Vitamin E	0.70 IU

USDA National Nutrient Database for Standard Reference (2004)

Table 2 Walnut oil composition

Lipids		Amount in 100 g of kernel		
Fatty acids, total			62.23 g	(100%)
Saturated, total			6.13 g	(10%)
Palmitic		16:0	4.40 g	(7%)
Stearic		18:0	1.66 g	(3%)
Ecosanoic		20:0	0.06 g	(<1%)
Monounsaturated, total			8.93 g	(14%)
Gadoleic		20:1	0.13 g	(<1%)
Oleic		18:1	8.80 g	(14%)
Polyunsaturated, total			47.17 g	(76%)
Linoleic	(Omega-6)	18:2	38.09 g	(61%)
Linolenic	(Omega-3)	18:3	9.08 g	(15%)

USDA National Nutrient Database for Standard Reference (2004)

walnuts (8–13) replaced approximately 32% of the energy from monounsaturated fat. The walnut diet increased endothelium-dependent vasodilation by 64% and reduced vascular cell adhesion molecule-1 by 20%. The diet also decreased total cholesterol and LDL cholesterol. Just recently (Reiter et al., 2005), a significant level of melatonin was identified in walnuts. According to the author R.J. Reiter, "the ingredients in walnuts would be expected to reduce the incidence of cancer, delay or make less severe neurodegenerative diseases of aging . . . and reduce the severity of cardiovascular disease."

2 Botany

2.1 Taxonomy

The family Juglandaceae consists of seven genera and about 60 species of deciduous, monoecious trees with alternate, pinnately compound leaves. It has been extensively studied by Manning (1978) and Manos and Stone (2001). In addition to the genus *Juglans* (walnuts), the family includes *Carya* (pecans and hickories), *Pterocarya* (wingnuts), *Platycarya*, *Engelhardia*, *Alfaroa*, and *Oreomunnea*.

Members of the genus *Juglans* are trees or large shrubs possessing twigs with chambered piths, large aromatic compound leaves, generally solitary staminate catkins on 1-year-old wood, and female flowers on current season's wood. The husked fruit is a false drupe containing a large, woody-shelled nut. All *Juglans* produce edible nuts, although size and extractability differ considerably. Most species are highly regarded for their timber.

The genus *Juglans* consists of approximately 21 species native to parts of North America, the Andean region of South America, and the mountain ranges traversing Central Asia (Table 3). These species have been grouped taxonomically into four sections: *Juglans*, *Trachycaryon*, *Cardiocaryon*, and *Rhysocaryon*.

Section Juglans. The *Juglans* section consists solely of the commercially valuable Persian or English walnut, *J. regia*. This section is characterized by a four-celled nut, a husk that separates from the nut at maturity, and seedlings with two rows of buds immediately above the cotyledons and below the spirally arranged compound leaves. The typically large tree grows to a height of about 30 m and produces large, relatively smooth, and generally thin-shelled nuts (Fig. 1).

J. regia selections have been identified in which nuts vary from nearly round to the greatly elongated 'Barthere' and from pea sized to more than 5 cm diameter. Trees with a weeping growth habit have been identified in Belgium and California, and variations in leaf morphology and color have been identified. Cutleaf types include 'Heterophylla' and 'Laciniata.' 'Monophylla' has leaves with only an enlarged terminal leaflet occasionally with two greatly reduced side leaflets; 'Adspersa' produces mottled white leaves, and 'Purpurea'

Table 3 Species and their range in the genus *Juglans* (after Manning 1978)

Section and species	Common name	Range
Juglans		
J. regia L.	English or Persian walnut	Southeastern Europe, Iran to Himalayas, and China
Trachycaryon		
J.cinerea L.	Butternut	Eastern United States
Cardiocayon		
J. ailantifolia Carr. (*J. sieboldiana*)	Japanese walnut	Japan
var. *cordiformis*	Heartnut	Japan
J. cathayensis Dode	Chinese walnut	Eastern China, Taiwan
J. mandshurica Maxim.	Manchurian walnut	Manchuria, northeastern China, Korea
Rhysocaryon		
J. australis Griseb.		Argentina
J. boliviana (C. DC) Dode		Western South America
J. californica S. Wats.	Southern California black walnut	Southern California
J. hindsii (Jeps.) Rehder	Northern California, black walnut	Northern California
J. hirsuta Mann.		Northeastern Mexico
J. jamaicensis C. DC.	West Indies black walnut	West Indies
J. major (Torr. Ex Sitsgr.) Heller	Arizona black walnut	Southwestern United States, northwestern Mexico
var. glabrata Mann.		South-central Mexico
J. microcarpa Berl. (*J. rupestris*)	Texas black walnut	Southwestern United States, northwestern Mexico
var stewartii (Johnston) Mann.		Northern Mexico
J. mollis Englem. Ex Hemsl.		Central Mexico
J. neotropica Diels		Northwestern South America
J. nigra L.	Eastern black walnut	Eastern United States
J. olanchana Standl. and L.O. Williams		Guatemala
var. standleyi Mann.		Southeastern Mexico
J. pyriformis Liebm.		Southeastern Mexico
J. soratensis Mann.		Bolivia
J. steyermarkii Mann.		Guatemala
J. venezuelensis Mann.		Venezuela

Fig. 1 Walnuts on tree

exhibits leaves of a dull red color (Rehder, 1940). Cultivars with bright red seed coats have also been bred (McGranahan and Leslie, 2004).

The considerable variation within *J. regia*, particularly in nut size and shape, led taxonomists to describe six additional species that others have not accepted but which illustrate some of the diversity (Dode 1909). *J. sigillata* Dode, a type from southern China and Tibet with a very thick rough-shelled nut, an adherent hull, and very dark colored kernels, is the most distinctive of the variations described and is currently accepted as a separate species by some botanists. This status has been supported by recent isozyme analysis. Known locally as the iron walnut, this type or species has been cultivated for a long time in Yunnan Province for its oil, and several cultivars have been developed.

Section Trachycaryon. The Trachycaryon section consists only of *J. cinerea* L., butternut, a North American species, characterized by a two-chambered nut exhibiting eight prominent ridges on the shell and an indehiscent husk. The

seedlings exhibit few if any scale buds immediately above the cotyledons, resulting in a long-naked area on the lower seedling stem where other species typically produce scale leaves. The nuts are borne in clusters of several nuts each on a long stalk, and the husks are conspicuously four-ribbed. Section Trachycaryon appears to be very closely related to the Asian section Cardiocaryon.

J. cinerea is native from New Brunswick to Georgia and west to Minnesota and Arkansas and is the most cold hardy of the North American walnuts. Also known as the white walnut or oil nut, this species is often found on river bottoms in mixed hardwood forests and will tolerate a high water table. Seldom found in pure stands and reaching a height of up to 30 m, it is a shorter, more spreading tree than *J. nigra* with which its range substantially overlaps. *J. cinerea* also has a relatively short lifespan, seldom living longer than 80–90 years.

Butternut wood is not as strong or durable as that of black walnut but is used for furniture, box, and toy construction. The kernels are large and in selected cultivars can be cracked out in halves. About 25 butternut cultivars have been selected from the native seedling population for their cracking characteristics, shell thinness, and yield. A few of these are cultivated, but no significant commercial use has developed and now butternut canker is decimating native stands.

Section Cardiocaryon. The Cardiocaryon section contains species that produce two-chambered nuts with 4–8 prominent ridges and indehiscent husks, and the nuts are borne in racemes of 5–25 nuts each. Seedlings exhibit five rows of scale buds immediately above the cotyledons, which merge into small alternate compound leaves higher on the stem. Members of this section are native to eastern Asia where their nuts and timber are utilized, but their susceptibility to walnut bunch disease has limited their horticultural development in the eastern USA.

J. ailantifolia Carr., the Japanese walnut reaches a height of 25 m, has leaves that are very pubescent on the lower surface, and bears its nuts in long racemes of up to 20 nuts each. This species is native to Japan where trees are generally found along streams and in moist plains. Although nuts of *J. ailantifolia* are typically difficult to crack, a seedling variant known as the heartnut, *J. ailantifolia* var. *cordiformis* (Maxim.) Rehd., bears heart-shaped nuts that crack more easily and from which kernels can be removed whole.

J. mandshurica Maxim., the Manchurian walnut, can grow to 30 m in height and 50 cm in diameter. Nuts are borne in clusters of five to seven nuts each on short, 10–15 cm, pendulous racemes. This species, native to northeastern China, Manchuria, and Korea, is the most cold-hardy of the *Cardiocaryon* but the nuts are difficult to extract. *J. mandshurica* is used in China mainly for timber and furniture and as a rootstock in cold areas of northern China. The nuts are highly variable in size and shell thickness.

J. cathayensis Dode, the Chinese walnut or Chinese butternut, is a vigorous tree or shrub up to 25 m in height. The small, edible nuts with very hard shells are produced on pendulous or erect spikes 8–15 cm long, which bear 6–13

flowers each. This species is native to much of central and eastern China and is thought to be botanically very close to *J. mandshurica*, perhaps a geographical variant, *J. cathayensis* var. *formosana* Hayata, native to Formosa and the southern portions of the *J. cathayensis* range, exhibits a smoother shell. *J. cathayensis* is reportedly a commonly used rootstock in regions of China along the Yangtze River.

Section Rhysocaryon. The Rhysocaryon section consists of approximately 16 North and South American *Juglans* species all of which exhibit four-chambered nuts with indehiscent husks, sutures that are not widened or winged, and shells that are ridged or striate but not completely smooth. Five rows of scale leaves at the base of seedling stems merge into small, spirally arranged compound leaves farther up the stem. *Juglans* species belonging to the section Rhysocaryon are found in much of the eastern USA and in localized portions of the west and southwest. Latin American members are found mostly in the mountains of Mexico, Central America, and the Andean region of South America with little geographical overlap of species ranges (Manning 1978). The species of this section are so closely related that it is often difficult to distinguish them, and generally nuts are so similar that they are of little value in separating species. Incomplete collection, considerable loss of the material that has been collected, and difficulty to travel in many of the remote mountainous areas where these trees are native have seriously impeded the study of both the taxonomic and economic characteristics of these species.

J. nigra L., the eastern black walnut, is the largest of the North American walnuts, reaching a height of 45 m and a trunk diameter of 2 m. It is native to the deciduous forests of the eastern USA and Canada where it is found most frequently in mixed stands on bottomlands and lower slopes with moist, well-drained soils. Eastern black walnut bears nuts with hard, black shells and stronger flavored kernels than those of *J. regia*. The irregular grooves and ridges on the shell separate it from the other species native to the USA, which produce evenly grooved to nearly smooth nuts.

Among wild *J. nigra* seedlings, there is considerable genetic variation in nut quality, blooming date, leafing date, age of first bearing, and growth rate. Over 400 cultivars of *J. nigra* have been selected for yield, nut characteristics, and timber quality. The best cracking black walnuts are the single-lobed sports, sometimes called peanut type, which have only a half nut and can be extracted whole. Examples include 'Throp', 'Blaettner', and 'Worthington'. Selections exhibiting lateral bud fruitfulness and up to 36% kernel have also been reported. 'Deming Purple' has reddish shades in its foliage, and a purple pellicle and 'Laciniata' is a variant with deeply indented foliage.

The high-quality wood is prized for furniture and gunstocks and is the most valuable hardwood produced in the USA. Eastern black walnut is now planted in Europe as both a timber species and a rootstock. The nuts, used for candies, baked goods, and ice cream, are scarce and generally more expensive than those of the Persian walnut. Almost all eastern black walnuts are harvested

from wild trees without the aid of mechanical harvesting, and kernel yield averages only 8–25%.

J. hindsii (Jeps.) Rehder, the northern California black walnut, was once considered a variety of. *J. californica* S. Wats., but is now considered a separate species. The tree grows to a height of 30 m and produces round, smooth, hard-shelled nuts which vary considerably in size and quality but are generally smaller and less strongly flavored than those of *J. nigra*. At the time of European settlement, *J. hindsii* existed in only a few small groves in northern California. Although the species is a common shade tree in the region now, many of the existing trees may be hybrids with other black species. The most important use of *J hindsii* is as a rootstock alone or in the hybrid rootstock Paradox (*J. regia*×*J. hindsii*).

Other species in the Rhysocaryon section may be important locally and some have been tested as rootstocks to a limited degree. Recent work on the Paradox rootstock suggests that the Paradox rootstock commercially available may have hybridized with black species other than *J. hindsii*.

Other genera. Among the other genera of Juglandaceae, only the wingnuts (*Pterocarya*) have shown any promise of contributing to *Juglans* production. *Pterocarya stenoptera* C. DC., a vigorous colonizer of river banks and moist alluvial soils in its native China, exhibits a number of desirable rootstock properties, including considerable tolerance to *Phytophthora*, waterlogging, and nematode damage, but is incompatible with some cultivars of *J. regia* (McGranahan and Catlin 1987).

2.2 Interspecific Hybrids

Many of the species of this genus are capable of hybridizing with each other. In general, the black walnuts of section Rhysocaryon will not cross with species of sections Trachycaryon or Cardiocaryon, but *J. regia* will cross, at least to some extent, with members of the other three sections. The ability of *J. nigra* to cross with *J. ailantifolia* is an apparent exception to this generalization. The hybrid of greatest commercial importance is *J. regia*×*J. hindsii,* known as 'Paradox' and covered under the rootstock section of this chapter. Royal hybrids (*J. hindsii*×*$J. nigra*) are less vigorous than Paradox, perhaps due to their crop load, and are not used as rootstocks. Other *Juglans* hybrids with named cultivars include *J. cinerea*×*J. ailantifolia* crosses known as 'butterjaps' or 'buartnuts' and a *J. nigra*×*J. ailantifolia* cross named 'Leslie Burt', which exhibits anthracnose resistance. Although the native ranges of *J. nigra* and *J. cinerea* overlap substantially, the absence of confirmed hybrids suggests that these species are intersterile. In China, hybrids of *J. regia*×*J. mandshurica*, formerly the species *J. hopeiensis* Hu, are native to northern Hebei province near Beijing in northeast China.

2.3 Reproductive Biology

All *Juglans* species examined have 32 (2*n*) chromosomes and are monoecious, i.e., the male and female flowers are borne separately on the tree. The male flowers are densely packed on catkins that hang from the tree in the spring. Each catkin has up to 40 sessile petaless florets each with numerous stamens. The immature naked catkin buds first appear in leaf axils in late summer and persist over winter maturing in the spring in the axils of leaf scars on wood from the previous season. Female flowers are borne on current season's growth in spikes of two (to five) flowers in *J. regia* and more in other species. Flowers are typically produced on the tips of terminal shoots shortly after leaves emerge (Fig. 2). In some cultivars, female flowers are also produced on the tips of lateral shoots. This type of flowering is termed 'lateral bud fruitfulness' and is associated with high yields when trees are young. Lateral buds are rare on mature trees. The female flower consists of a hairy involucre fused to four sepals enclosing the pistil, which has a swollen base, the ovary, and a short style with a forked stigma with two feathery stigmatic lobes. The ovary is surrounded by the ovary wall, and inside the single locule is divided into four parts by the

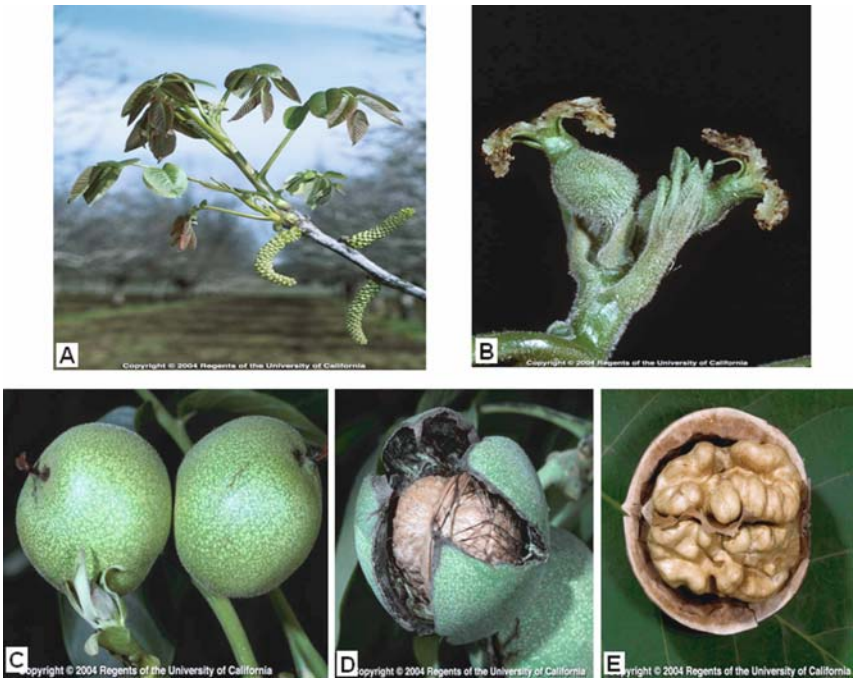

Fig. 2 (**A**) Walnut female flowers and catkins (male flowers); (**B**) flowers after pollination; (**C**) mature nuts in hull; (**D**) harvestable nuts; and (**E**) nut in shell

major and minor septa. Attached to the ovary is a single ovule enclosed by a single integuement (Polito 1998; Pinney et al. 1998).

Walnuts are heterodichogamous with male and female flowering occurring at different times. Some cultivars are protandrous, with the male flowering first, while others are protogynous, with the female flowering first. There is usually some overlap between male and female bloom, and walnuts are self-fertile, but the dichogamy promotes outcrossing.

Flowering occurs in the spring from mid-April for early cultivars to mid-May for late-blooming cultivars. Pollen is wind-borne and can be carried great distances, but is relatively short-lived. The female is receptive when the two stigmatic surfaces separate to form a V-shape and secrete an exudate that makes them appear moist. Once the lobes have achieved a 45-degree angle, the surface begins to dry, and the female flower is no longer receptive. If it has not been fertilized, the flower will expand for the next 3 weeks to the size of a marble and abscise. The stigma surfaces stay moist if the flower has not been fertilized. Another cause of pistillate flower abscission (PFA) is caused by excess pollen (McGranahan et al. 1994a). In this case, the flowers shrivel and abscise shortly after they lose receptivity. PFA occurs to some extent in all cultivars but is most serious in the cultivar 'Serr'. The nut is harvestable approximately 19–22 weeks after bloom.

Timing of leaf emergence and harvest has always been an important consideration in selecting walnut cultivars. Early-leafing cultivars are more susceptible to frost and, in California, to walnut blight and insect problems. But early-leafing and flowering cultivars usually harvest early (in September) and have the advantage of early entry into the holiday nut trade. Late-harvesting (mid-October) cultivars can be subject to difficulties such as rain, which can interfere with harvesting operations. Vigorous late-harvesting cultivars that continue growing late into the autumn may also be more prone to injury by early frosts and winter injury.

3 Breeding

3.1 Objectives

The major breeding objectives are to increase yield, quality, and range of harvest dates while decreasing the amount of chemical input required to control pests and diseases (Table 4). The ideal walnut cultivar would be relatively late leafing to escape frost and the rains that spread walnut blight (*Xanthomonas campestris* pv. *juglandis*), precocious (yielding more than 500 kg/ha in the fourth year), and vegetatively vigorous with bearing on both terminal and lateral shoots. It would have a low incidence of PFA and other drops and would not be alternate bearing. It would have high production capacity (>6 MT/hectare) with low chemical input required. The harvest season would end in early October. The nutshell would be relatively smooth, well sealed, and make up no more than 50%

Table 4 Pests and diseases of walnut. (For more information see Ramos (1998) or IPM (2003))

Common name	Causal agent	Parts affected	Symptoms and signs	Importance	Control
Bacterial diseases					
Walnut blight	*X. campestris* pv. juglandis (Pierce) Dye	Leaves, shoots, husk, nuts	Spots (coalescing)	Major, widespread	Copper spray
Crown gall	Agrobacterium tumefaciens (Smith & Townsend) Conn	Roots, crown	Galls	Major	Sanitation, gall removal
Deep bark canker	*Brennaria rubrifaciens* Wilson et al.	Trunk	Cankers	Minor	Rootstock selection
Shallow bark canker	*Erwinia nigrifluens* Wilson, Star & Berger	Trunk	Cankers	Minor	
Fungal diseases					
Anthracnose	*Gnomonia leptostyla* (Fr.) Ces. et de Not (*Marssonina juglandis*) (Lib.) Magn. (anamorph)	Leaves, shoots, husk, nut	Spots (coalescing)	Major but not in California	Copper spray
Armillaria root rot	*Armellaria mellea* (Vahl:Fr.) *P. Kumm.*	Roots, crown	Rot, mycelial fans rhizomorphs	Minor, severe in some locations	Sanitation
Phytophthora root and crown rot	*Phytophthora* spp.	Roots, crown	Rot, cankers	Major	Rootstock selection
Butternut canker	*Sirococcus clavigignenti-juglandacearum*	Trunk, branches	Cankers	Only in butternut	
Viral diseases					
Blackline disease	Cherry leafroll virus (CLRV)	Systemic in *J. regia*	Blackline at graft union between *J.*	Major	Tolerant rootstocks

Table 4 (continued)

Common name	Causal agent	Parts affected	Symptoms and signs	Importance	Control
			regia and hypersensitive rootstocks		
Nematodes					
Root lesion nematode	*Pratylenchus vulnus* Allen & Jenson	Roots	Black lesions on roots, dead feeder roots	Major	
Root knot nematode	*Meloidogyne* spp.	Roots	Swellings on roots	Limited to coarse soils	Sanitation, soil fumigants
Ring nematode	*Mesocriconema xenoplax* (Raski) Luc and Raski	Roots	Stunted feeder roots	Minor	Sanitation, soil fumigants
Insects					
Codling moth	*Cydia pomonella* L.	Husk, nut	Premature abscission, frass at entry hole	Major, increases susceptibility to navel orange worm	Insecticide sprays, pheromone mating disruption predators and parasites
Navel orange worm	*Amyelois transitella* Walker	Husk, nut	Wormy nuts, frass inside nut	Major	Prompt harvesting, insecticide sprays
Walnut husk fly	*Rhagoletis completa*, Cresson	Husk	Wormy husk, shell stains	Major	Insecticide sprays
Mites, aphids, and scales					
Walnut aphid	*Chromaphis juglandicola*, Kaltenbach	Leaves	Feeding aphids, leaf abscission	Minor with flare ups	Parasitic wasp: *Trioxys pallidus*

of the nut weight. The nuts would fit the category of large or jumbo. The kernel would be plump and light colored, weighing about 8–9 g, and come out easily in halves. The tree would be at least moderately resistant to pests and diseases.

3.2 Genetic Resources

A breeding program depends in part on a diverse collection of germplasm as a source of raw material from which traits of interest can be identified (McGranahan and Leslie 1990). For the past two decades, extensive evaluations of seedlings in orchards and naturalized trees have been undertaken in the Mediterranean countries of Europe and to a lesser extent North Africa (see Proceedings of Walnut Symposia: Acta Horticulturae numbers (1990) **284**, (1993) **311**, (1997) **442**, (2001) **544,** and 2006 (705)). From this work, several new cultivars have been identified (Tomas 2000). China has also had an active nationwide search for new cultivars from seedling orchards. Because Persian walnuts are native to the mountains of Central Asia, considerable effort in the USA has been directed toward collecting material from that area (Leslie and McGranahan 1998). Funding and participation in this work have included a century-long plant introduction endeavor by United States Department of Agriculture (USDA) plant collectors, and more recent trips by USDA and university researchers. Collecting has been funded in part by California growers, USAID exchanges, and USDA-ARS Germplasm exploration funds. Material has also become available for use through international germplasm exchanges, private breeders, hobbyists, customs confiscations, and observant growers in the state who have noticed interesting seedling trees.

A very useful book, "Inventory of Walnut Research, Germplasm and References," has recently been published by FAO and it describes a great number of germplasm collections in the world, especially in the European Union (Germain 2004). In the USA, both the University of California (UC) and the USDA National Clonal Germplasm Repository, Davis, California (NCGR-Davis), maintain walnut germplasm collections. The content at the Davis Repository walnut collection is listed at www.ars.usda.gov/main/www.ars-grin.gov/dav_main.htm?modecode = 53-06-20-00.

The intent of the USDA collection is to include as broad a diversity of all walnut species as possible and maintain it for public distribution of material. It will not accept proprietary material and is managed primarily for wood and nut distribution to researchers worldwide. The UC Davis collection includes a representation of California commercial varieties, advanced selections, and some proprietary material, and is focused primarily on material of interest for breeding purposes (Tulecke and McGranahan 1994). It is managed for a variety of activities, including crossing, breeding evaluations, and graftwood distribution of advanced selections. While there is some overlap of material, duplication is generally avoided, and the two collections are used cooperatively.

3.3 Evaluation

Germplasm in these collections has to be evaluated and characterized to determine its useful attributes. Descriptors for evaluating germplasm have been published (McGranahan et al. 1994b) by the International Bureau of Plant Genetic Resources (formerly International Plant Genetics Resources Institute). The *Inventory of Walnut Research, Germplasm and References* (Germain 2004) includes evaluations of the primary international cultivars. The USDA Germplasm Resources Information Network (GRIN) has descriptions of some of the germplasm held at the NCGR (www.ars-grin.gov/). Evaluation of the UC Davis collection is primarily for the UC Davis breeding program, and the traits evaluated are shown (Table 5). In addition, the UC Davis collection has been evaluated for allergenic proteins to determine whether a nonallergenic walnut could be bred, but all the germplasm contained allergenic proteins (Comstock et al. 2004). Susceptibility to *Aspergillus flavus* and aflatoxin contamination was also evaluated. Susceptibility was comparatively low in walnuts compared to other nut crops, but there was significant variation when artificially inoculated; for example, 'Chico' had a much higher level of aflatoxin than 'Tulare' (Mahoney et al. 2003). Both stem-end hole size and degree of hull pubescence were evaluated to determine their effects on codling moth. Both traits showed significant variation, but only pubescence affected the codling moth by slowing its movement across the hull, allowing predators more time to kill the larvae. However, it was determined that no germplasm was sufficiently pubescent to have a major impact on codling moth infestation (unpublished data).

Table 5 Cultivar traits under evaluation and estimated heritabilities (Hanche et al. 1972)

Field	h^2	Crack out	h^2
Leafing date	0.96	Shell texture	
Female bloom: first, peak, and last	0.93	Shell color	
Male bloom: first, peak, and last	0.8	Shell seal	0.38
Dichogamy		Shell strength	
Percent overlap: male and female		Shell integrity	
Catkin abundance		Shell thickness	0.91
Female flower abundance		Packing tissue thickness	
Percent fruitful laterals	0.39	Nut weight	0.86
Yield	0.07	Kernel weight	0.87
Blight		Percent kernel	
Codling moth		Fill	
Sunburn		Plumpness	
Harvest date	0.85	Ease of kernel removal	
		Color (extra light, light, light amber, amber)	0.52
		Shrivel	
		Veins	0.49

Heritabilities are high for many traits of interest (Hanche et al. 1972; Forde and McGranahan 1996) (Table 5). However, it has been shown that many traits change with clone age; for example, leafing out, bloom, and harvest date all shift to 2 weeks earlier, stabilizing at age 15. Shells also thicken and seals improve, but the in-shell weight, kernel weight, and percent kernel all decrease (McGranahan and Forde 1985).

3.4 Crossing Methods

The UC breeding program has used two distinct procedures for crossing parent material. In the first method, wind-blown pollen is excluded from female flowers of interest by covering them with tightly secured bags that have small plastic windows. Pollen is collected from the other parent of interest and stored frozen over saturated magnesium chloride until use. When bagged female flowers open and are receptive, pollen is applied through the bags with a hypodermic needle. Bags are later removed and nuts marked for collection in the fall. The male parent is known with this method, but the costs are high and seedling production is low.

The second method is to locate geographically isolated young trees of the desired female parent. Using young trees is important, because as a cultivar matures, the female flowers are usually present 2–3 years before the male flowers. This often requires the cooperation of a grower with a recently planted orchard. Any male flowers on these trees are removed by hand before bloom to prevent selfing. Once the female flowers begin to bloom, pollen of the desired male parent or parents is applied by airbrush several times during the bloom period. At harvest, the cooperating grower either donates or is compensated for the nuts. This method produces many more seed at lower cost but with low certainty of the male parent. Male parents of selections can be determined later by DNA analysis. Some selfing also occurs, which results in stunted, twisty trees with russetted hulls and small kernels.

3.5 Seedling Evaluation

Seed collected from these crosses is then stratified and grown to produce the next generation of seedlings. These are screened as they mature for traits of interest (Forde and McGranahan 1996). Commercial walnut nurseries have generously donated growing ground, time, resources, and expertise to assist this aspect of the program.

After 1 year in the nursery, trees are dug and replanted on wider spacing for evaluation. At this stage, trees are grown on their own roots, not grafted to rootstock. Most commonly, these trees are planted on UC Plant Sciences Department growing grounds and farmed by department staff supported by

university and grower funding. In some cases, growers have assisted the program by donating orchard space for this purpose and have farmed these trees during the evaluation process. This has been done by planting between rows in an existing widely spaced orchard, or more effectively, by interplanting in available open space in a newly established orchard and then removing the breeding program trees as evaluations are completed and the grower's orchard matures to fill the canopy.

As seedling trees mature, they are evaluated in the field for traits of interest, including leafing, flowering, and harvest dates, yield, and growth habit (Table 5). When the trees are grown in university orchards, they are left unsprayed so that variation in resistance to insects and disease can be observed. When grown within commercial orchards, this is not normally possible. Nut samples are hand collected from each tree at maturity. Samples are dried, cracked by hand, and evaluated for percentage of kernel, kernel quality, kernel weight, shell characteristics, and yield of halves (Table 5). Data are entered into a database and summarized for multiple years. In addition, samples of promising individuals are sent to commercial processors for their independent evaluation.

Collected data are presented to farm advisors, growers, and nurserymen in several ways. The first is at the annual Walnut Research Conference as part of the Walnut Improvement Program's annual report. Data on selections are presented orally to attendees and published in the annual proceedings of the conference (available from author).

The breeding program also holds an annual "Crackout Meeting" in the spring attended by farm advisors, handlers, nurserymen, and growers. Growers, handlers, and nursery attendees generally have an expressed interest in development of new varieties, are interested in assisting with evaluation of material, or are otherwise active in research activities and the marketing board. At this all-day meeting, the data reports are distributed, and kernel samples and intact nuts of the material under evaluation are displayed. Attendees are asked to review the material, examine the samples, and provide written comments. In an ensuing discussion period, they provide valuable input on priorities from their varying perspectives, help rank material, and suggest which should continue in the program. The program also regularly invites interested parties to view selections in the field, either through a formal field day or by scheduling informal visits at their convenience. Progress in the program and information about selections are also presented periodically to a wider range of growers at annual county grower meetings held around the state.

3.6 Selection Trials

Once an individual seedling shows promise and is selected for further trials, graftwood is collected from the original seedling and grafted to rootstocks.

Nurseries have often provided assistance at this stage by donating rootstock, supplying grafters, and, in many cases, growing the grafted trees for the program.

Grafted trees of each selection are then planted in test blocks on orchard spacing at diverse locations for further evaluation. Currently, these tests blocks are located at the Chico State University Farm in the northern part of the state, on the UC Davis campus in the central region, and at the UC Kearney Field Station in the south. These blocks are managed by cooperative extension farm advisors and are used to evaluate the performance of selections on rootstocks under a wide range of conditions, obtain a better look at yield, and allow farm advisors and growers to see selections in their local area.

In addition to the university plots, interested growers around the state have volunteered to establish trials ranging in size from several trees to several acres. Farm advisors assist in identifying suitable growers, establishing plots, and observing performance. Graftwood is distributed to these growers under test agreement, and they are asked to participate in its evaluation and to attend the *crack out meeting*. This gives the program valuable input on performance under a variety of conditions and in commercial settings from observers with extensive experience. Growers feel they are assisting the process and get an early look at the material that is most interesting for their situation.

As new selections begin to show promise, commercial nurseries are encouraged to acquire graftwood from the program to test the varieties for themselves and to begin increase blocks of their own. This ensures that nurseries have adequate input into final selection, firsthand knowledge of the material, particularly of its grafting performance, and growth habit and training requirements and build an adequate supply of production wood by the time the new variety is released. As with grower trials, nurseries receive wood under test agreement. This allows them to propagate for testing purposes, including grower trials, but trees cannot be produced for sale until they are patented.

3.7 Release of Selections

Selections that continue to show promise in test blocks and grower trials become candidates for patent and release as new cultivars. The patent disclosure process requires an extensive description of the selection, a summary of available data, and identification of attributes distinct from existing varieties.

Once a selection is patented as a new cultivar, nurseries may obtain a commercial license from the University of California that allows sale of trees. A per-tree royalty is assessed at the time of sale from the nursery and returned to the university. After patenting costs are recovered, part of this fee is assigned for overhead, and part is returned to the breeding program as well as the breeders.

Patenting provides a return to the inventor and the university but also seeks to protect the growers from unlimited distribution. Patented material is not allowed to be sold or grown outside of California for 5 years after release. After that period, overseas licensing provides a return to the program that would not otherwise occur.

3.8 Backcross Breeding for Hypersensitivity

Marker-assisted backcross breeding is being used to develop a commercial quality, *J. regia*-like cultivar with resistance (hypersensitivity) to the CLRV, which causes blackline disease (Woeste et al. 1996). We showed that a single dominant gene from *J. hindsii* confers hypersensitivity and that progeny from backcrosses (*J. hindsii* × *J. regia*) × *J. regia* segregates 1:1 hypersensitive–to-lerant (McGranahan et al. 1997). Currently we are evaluating the BC4 generation. An anomaly in all the backcrosses is that they are male sterile, i.e., catkins, if formed, abscise when immature. We have selected three backcross genotypes, with close to commercial quality, for field trials. The field trials are designed to determine whether CLRV-infested pollen infects a hypersensitive flower, whether any damage to the flowers occurs at fertilization, and whether nut set is affected.

3.9 Breeding Accomplishments

Prior to the Serr-Forde breeding program (1948–1978) in California, most cultivars grown in Northern California, where the industry now resides, were cultivars brought from France by Felix Gillet in the late 1800s or chance seedlings. Gene Serr and Harold Forde made remarkable progress in breeding new cultivars that revolutionized the industry. Their primary breeding objectives were to combine the late leafing and quality of the French types with the lateral fruitfulness and precocity of 'Payne'. They made 196 crosses, evaluated about 6000 progeny, and released 13 cultivars, 10 in 1968 and 3 in 1978. The most important of these are 'Vina', 'Serr', 'Howard', and 'Chandler' (Ramos, 1998). In 1993, 'Tulare' was released from a cross made 27 years earlier by Serr and Forde (McGranahan et al. 1992).

Recently, four new cultivars have been released. 'Robert Livermore' is a red-skinned walnut (McGranahan and Leslie 2004). 'Sexton', 'Gillet', and 'Forde" (patent pending) are all precocious in bearing, laterally fruitful, high yielding, midseason harvesting, with low blight scores and high quality kernels. The latter two are protogynous, which is unusual in the cultivars available

4 Rootstock Improvement

The rootstock is the other half of the tree and provides anchorage, absorption of water and nutrients, hormone synthesis, and storage. Rootstocks are more difficult to study because they are mostly underground, and rootstock improvement is developing slowly because clonal propagation has not yet been commercialized in California. Traits of common rootstocks are shown in Table 6. Clearly genetic improvement is needed. To date, the Paradox rootstock (*J. hindsii* × *J. regia*), which exhibits hybrid vigor, is superior to pure species in most traits, but many other species combinations have not been tested (McGranahan and Catlin 1987). Paradox is seed propagated from *J. hindsii* (northern California black walnut) trees that are naturally pollinated by *J. regia* pollen.

Blackline tolerance. In California, we have approached the blackline problem, caused by the CLRV, through both cultivar hypersensitivity and rootstock tolerance. The latter is aimed at developing a rootstock combining the *J. regia* response to blackline disease with the vigor and other attributes of Paradox. This can be achieved, in theory, by selecting vigorous, tolerant individuals among seedlings of a backcross generation *(J. hindsii* × *J. regia)* × *J. regia*. In 1988, 13,000 Paradox offspring from 17 source trees were planted in a randomized complete block design with six blocks in *Phytophthora*-infested soil. Between 1992 and 1994, they were screened for vigor and tolerance to the virus. Five seedlings were selected in 1994, but it has taken until last year to establish grower trials to compare their performance in the field to Paradox and *J. regia* rootstocks because of the challenges of clonal propagation.

Nematode, crown gall, and Phytophthora resistance. A study to evaluate the diversity of Paradox rootstocks was initiated in 1996. It was designed to examine variability among families of commercially available Paradox seedlings and controlled crosses between different black walnut species and *J. regia*. Eleven California walnut nurseries each donated about 500 seeds from each of three Paradox-producing black walnut source trees each year for 2 years. These were planted in replicate blocks in three nurseries, measured and divided into subsets. Four subsets were planted and grafted as orchard trees (Wilbur Reil, Bob Beede, Joe Grant, Richard Buchner); two subsets were screened for nematode (*P. vulnus*) resistance by Michael McKenry (unpublished), and two were screened for crown gall (*A. tumefaciens*) resistance (McKenna and Epstein 2003). Two subsets of ungerminated seed were provided to Greg Browne for *Phytophthora* screening (Browne et al. in press).

The work is ongoing in the four long-term field trials, but in the process of screening seedlings for various traits, it became apparent that certain individual seedlings were superior. Two genotypes from the crown gall screen were selected; one proved to be an escape rather than a resistant genotype, and the other remains to be retested. Greg Browne has identified several genotypes that continue to have low susceptibility to *Phytophthora citricola* in repeated screens

Table 6 Walnut rootstock response to pests and diseases

Rootstock	Crown gall	Phytophthora root and crown rot	Blackline disease	Armillaria root rot	Root lesion nematode	Root knot nematode	Salts
Persian walnut (*J. regia*)	Susceptible	Very susceptible	Symptom less	Susceptible	Very susceptible	Susceptible	Sensitive
Northern California black walnut (*J. hindsii*)	Susceptible	Very susceptible	Hypersensitive	Variable	Susceptible	Resistant	Less sensitive
Paradox walnut (*J. hindsii* × *J. regia*)	Very susceptible	Susceptible	Hypersensitive	Variable	Very susceptible	Unknown	Sensitive
Wingnut (*P. stenoptera*)	Resistant	Resistant	Hypersensitive	Susceptible	Tolerant	Unknown	Unknown

For more information on pests and diseases see Ramos (1998) or IPM (2003)

of micropropagated plants (Browne et al. in press). Mike McKenry found no resistance to nematodes but identified one genotype that did not appear to be affected by infestation (tolerant response). Most of the selected genotypes in this study have been micropropagated for field trials. These have been repropagated and are undergoing further field trials. It is expected that four or five new clonal rootstocks will be released from this study.

Much more work is needed on rootstocks. Since the hybrids appear to have the most vigor, it is important to evaluate the performance of different species in hybrid combinations. One that is readily available in South America and hybridizes easily with *J. regia* is *J. australis* from Argentina. Other possibilities are *J. neotropica* (northwestern South America) and *J. olanchana* (Mexico and Guatemala).

Clonal rootstock propagation. From the studies described above, it is clear that clonal rootstock is highly desirable. So far, only one commercial lab routinely produces micropropagated walnuts (Vitrotech Biotecnologia Vegetal, S.L., Murcia, Spain). Our lab has been successful in micropropagation, but we do not attempt it on a commercial scale. Micropropagation is accomplished by disinfestation and multiplication of nodal cuttings on gelled Driver-Kuniyuki-Walnut (DKW) medium (McGranahan et al. 1987). After initiation in vitro, shoots must be transferred frequently (two to five times per week) until the medium is no longer discolored by exudates. Multiplication occurs through axillary shoot proliferation and excision to initiate new cultures. Throughout the multiplication phase, walnuts have to be transferred relatively frequently. Cultures are maintained at room temperature under cool white fluorescent lighting.

A two-phase rooting system is used for micropropagated shoots (Jay-Allemand et al. 1992). Roots are induced by placing shoots on an auxin-containing medium in the dark. Induced shoots are then transferred to a vermiculite-gelled medium substrate and maintained in the light for 3 weeks. Rooted shoots are then transplanted to well-drained potting soil and acclimatized in a fog chamber in the greenhouse for 2 weeks, followed by a week or two under shade cloth. Dormancy can be induced once the plants have achieved 10–20 cm height by placing them at 10°C under short day length and low light intensity for 3 weeks. (Dormant plants can be stored for up to 6 months at 5°C.) Dormant plants are then planted in the nursery where they uniformly resume growth. A full description of the method will be published shortly (Leslie et al. in press).

5 Biotechnology

Many of the new tools of biotechnology have been applied to walnuts, as recently reviewed in Dandekar et al. (2005), but like many fruit and nut crops, walnuts lag behind the agronomic crops in this field. Gene transfer techniques

have been in use for walnuts since 1988 (McGranahan et al. 1988), and field trials of mature transgenic trees are under way. Genes of interest include Bt from *Bacillus thuringiensis* for insect resistance (Dandekar et al. 1998; Leslie et al. 2001) and crown gall silencing for resistance to crown gall (Escobar et al. 2002). Tree architecture has been modified by the *rolABC* genes from *A. rhizogenes*, but the goal of increasing rootability was not achieved (Vahdati et al. 2002). When used as rootstock, the smaller stature and compressed internodes of the rol trees did not effect the phenotype of the scion. The reticence of the public to accept genetically engineered organisms has prevented any commercialization of transgenic walnut trees, but it is expected that transgenic rootstocks will prove more acceptable.

Marker-assisted selection is being used successfully in a backcross breeding program designed to transfer hypersensitivity to CLRV from *J. hindsii* into a commercially acceptable Persian walnut cultivar. This tool greatly reduces the time required to screen progeny in each generation. DNA finger printing is becoming routine for cultivar identification (Dangl et al. 2005), and DNA sequence markers were used to identify the species involved in a large study of Paradox seedlings (Potter et al. 2002). Gene cloning from walnuts is under way, and genes of interest include those that code for tannin, naphthaquinone, unsaturated fatty acid, and flavonoid biosynthesis. In spite of activity in walnut biotechnology, genome mapping will only take place in the distant future, when the tools are readily available and less expensive than they are today.

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Note: The letters '*f*' and '*t*' following the locators refer to figures and tables respectively